Journal of
DAIRY SCIENCE

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PEOPLE AND EVENTS

UNIVERSITY NEWS

Dr. P. H. Tracy Honored by Friends and Associates

On July 29, Professor and Mrs. P. H. Tracy and daughter, Judith, were honored by 180 former students, associates, and friends at a dinner in the Illini Union at the University of Illinois. Professor Tracy is retiring from the faculty on September 1, after 39 years of distinguished service to the University and to the dairy industry in the state and nation.

Many of their friends could not join them for the evening, but their presence was felt in a bound volume of more than 225 letters which was presented to Professor Tracy as a permanent remembrance. As tangible evidence of their esteem, a gift of contributions of more than $1,400 was presented to the Tracys in the form of a certified check.

A very informal program followed the dinner with R. T. Milner, Head of the Department of Food Technology, presiding. He called on a few former students, associates, and industry leaders for extemporaneous remarks regarding Professor Tracy's career. The following responded: F. A. Russell, Emeritus Professor of Marketing, University of Illinois; E. L. Widemire, Sylacauga, Alabama, former World War I comrade and a friend of many years; O. F. Garrett, former student, colleague, and now Director of Research for M & R Dietetic Laboratory, in Columbus, Ohio; R. J. Ramsey, former student, colleague, and now President of Ramsey Laboratories, Cleveland, Ohio; Gilbert Gibson, former student and now with Sidney Wanzer & Sons Milk Company in Chicago, Illinois; E. B. Carter, Beatrice Foods Company, Decatur, Illinois, and President of Illinois Dairy Products Association. He presented Professor Tracy with a piece of luggage as a gift from the Illinois Dairy Products Association; C. N. Hansen, Vice-President and District Manager of Beatrice Foods Company, Champaign, Illinois; T. J. Kullman, Vice-President of Bowman Dairy Company, Chicago, Illinois; L. B. Howard, Dean of the College of Agriculture, University of Illinois. Dean Howard was the first head of the Department of Food Technology, of which Dr. Tracy is a member; G. Frederick Smith, Emeritus Professor of Chemistry, University of Illinois, President of the G. F. Smith Chemical Company, and President of Aeration Processes, Inc., Columbus, Ohio. Professor Smith presented Dr. Tracy with a replica of the aersol pressurized dispenser for whipping cream; J. H. Hetrick, former graduate student and now Director of Research for the Dean Milk Company, Rockford, Illinois.

Dr. and Mrs. Tracy looking at a volume of letters from associates and friends with Mrs. E. B. Mullins, a sister of Dr. Tracy, and Judith Tracy.

These people emphasized Dr. Tracy's activities as a teacher, researcher, consultant, and organizer. They may be summarized briefly.

Dr. Tracy made outstanding contributions. He pioneered investigations on homogenized milk, oxidized flavors in milk products, factors affecting the feathering of cream, the sanitary and structural properties of paper milk containers, and the use of corn sugar in milk products. He earned the Borden Award in 1946.

In addition to his work as a researcher, he contributed much as a teacher and adviser to undergraduates and graduate students. He is the author of a book on ice cream manufacture and senior author of a book on Dairy Plant Management. He has also advocated, and put into practice, new ideas for teaching courses in dairy technology, such as integrating processing and manufacturing of milk products into unit operations. He organized dairy technology courses in the industry by promoting the first in-service training course for plant personnel at the Bowman Dairy Company in Chicago in 1945. Nearly 400 people were enrolled in this first course. The establishment of the Department of Food Technology was Dr. Tracy's original idea. He assisted in the early organizational phases of this department which became a part of the College of Agriculture in 1947.

Dr. Tracy has been active in the affairs of the American Dairy Science Association. He

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(1) Standard Methods for the Examination of Dairy Products, ed. 10, New York, American Public Health Association, 1953. (2) Committee Report, Am. J. Pub. Health 42:1131 (Sept.) 1952. (3) Microbiological Methods, report at 66th Ann. Meet. Assn. Official Agricultural Chemists, Sept. 29, 1952: J. Assn. Official Agr. Chem. 36:91 (Feb.) 1953. (4) Standard Methods for Examination of Water, Sewage and Industrial Wastes, ed. 10, New York, American Public Health Association, 1955.

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served as Secretary of the Manufactures Section in 1933 and Chairman of this section in 1937. He served as Vice-President in 1946-47, President in 1947-48, and was a member of the Board of Directors from 1943 to 1950. He was Editor of the JOURNAL OF DAIRY SCIENCE from 1952 to late 1956. He made the JOURNAL more attractive, especially to industry, by introdueing the sections on People and Events and Our Industry Today.

Dr. P. H. Tracy, Mrs. Tracy, Dean L. B. Howard, Judith Tracy, Mrs. L. B. Howard.

Finally, Dr. Tracy found time to do much for the dairy industry as a lecturer, a consultant, and as an organizer. In 1930 he organized the Chicago Dairy Technology Society and was its Secretary for 25 years. This first society was the forerunner of 42 similar societies throughout the United States.

After September 1, Professor and Mrs. Tracy will make their home in DeLand, Florida, but their daughter, Judith, will remain in Urbana and enroll as a freshman in the School of Music at the University of Illinois.

Coulter Heads Minnesota Dept.

S. T. COULTER has been named head of the newly formed Dept. of Dairy Industries in the Institute of Agriculture of the University of Minnesota.

S. T. Coulter

This new department, to be located in the recently completed dairy industries building, is responsible for teaching, research, and public service in dairy manufacturing and dairy bacteriology.

Dr. Coulter has been on the Minnesota staff since 1925. A native of Weiser, Idaho, he received his undergraduate training at Oregon State College. He re-

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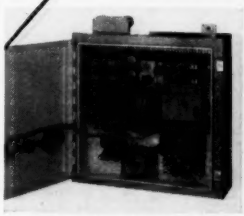

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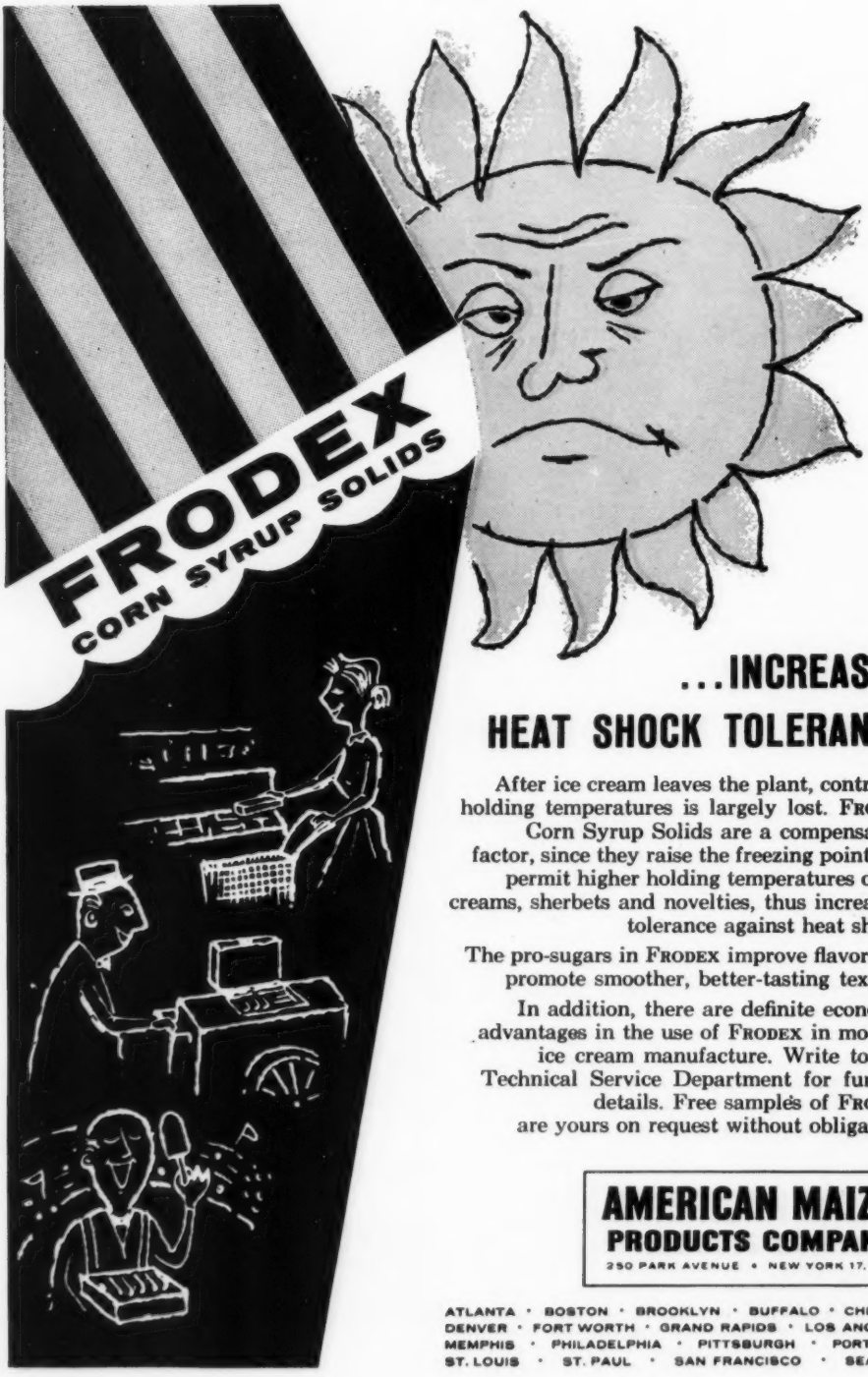

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ceived his M.S. and Ph.D degrees from Minnesota in 1930 and 1933, respectively.

Coulter is nationally known for research in dairy products, especially with butter and cheese, the development of powdered milk, and the production of dried milk.

In 1951 he received the Borden Gold Medal Award, one of the highest honors that can come to a dairy scientist. He has served on many committees of scientific and professional organizations, including A.D.S.A., the National Academy of Sciences, the National Research Advisory Board, and the American Dry Milk Institute. He also has served as consultant to national and United Nation's organizations.

L. H. Rich Retires

L. H. RICH retired as State Extension Dairyman of Utah in July. He has been Dairy Specialist since 1936, and was County Extension Agent of Utah County and Wasatch County ten years previous to coming to the college campus.

L. H. Rich

Professor Rich has been an active member of the American Dairy Science Association for 29 years since he earned the M.S. degree at the University of Minnesota.

He was a director of the American Dairy Science Association from 1952 to 1955, and has been chairman of Membership and Resolutions Committees, and also the DeLaval Awards Committee, in recent years. He was awarded the Superior Service Award by Secretary Ezra Taft Benson in 1956 at Washington, D. C., and was chosen for the DeLaval Extension Dairyman's Award in 1958.

Professor and Mrs. Rich travelled in the Middle East last year, with special interest in Egypt, Israel, Greece, and the dairy countries of Europe.

Professor Rich will be chairman of the Steering Committee at Utah State University to assist with the A.D.S.A. meeting in June of 1960, when the annual meeting convenes at Logan. He has been a tower of strength in extension activities in the Western States. His greatest achievement was the creation of a central system of processing Dairy Herd Improvement Records, whereby all calculations could be made in a central office with appropriate machines.

I. E. Parkin Receives Award

The Pennsylvania Dairy Sanitarians Association conferred upon Professor I. E. PARKIN

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This award climaxed almost 25 years of service to the dairymen of Pennsylvania. Parkin has pioneered in promoting milk sanitation with demonstrations in milking, milking machine care, cleaning, and sanitizing dairy equipment and, in particular, aiding in the promotion of bulk milk handling.

He was active in the establishment of five local dairy sanitarians groups. He also helped to establish from those groups the Pennsylvania Dairy Sanitarians Association which is the state affiliate of the International Association.

The citation and plaque were presented to Parkin at the annual banquet held at the University when the Pennsylvania Dairy Fieldmen's Conference was held there in July.

The plaque reads as follows: "For the unfaltering discharge and wise administration of his duties, for the recognition of his responsibilities, for his zealous interest and tireless devotion in the field of Dairy Sanitation and for his exemplary conduct as a sanitarian."

Recent Death

R. W. Brown, 71, research associate in the Dept. of Dairy Science, University of Saskatchewan, and former Professor and Head of the Dept. of Dairy Science at the University of Manitoba, died in the University Hospital, July 23.

Professor Brown graduated from the Ontario Agricultural College with a B.S.A. degree in 1913 and later obtained his Master's degree from Iowa State University, Ames. He was appointed to the staff of the Department of Dairy Husbandry at O.A.C. in 1913, served in this position for two years when he accepted a position in the Dairy Department at Iowa State. In 1917 he was appointed Professor of Dairying at the Manitoba Agricultural College at Winnipeg, Manitoba, and was Chairman of this Department until he retired in 1954. Early in 1955 he was appointed Research Associate at the University of Saskatchewan.

During his tenure at the University of Manitoba he guided and instructed over 700 dairy students. During his career he made numerous scientific contributions which contributed to the betterment of the dairy industry not only in Manitoba but nationally.

He was a life member of the Manitoba Dairy Association, member of the American Dairy Science Association, Agricultural Institute of Canada, the Honor Society of Phi Kappa Phi and contributing editor to the Canadian Dairy and Ice Cream Journal.

Professor Brown held the distinction of the longest appointment of any dairy professor in Canada, and over the years was one of the most active Canadian research workers in the field of butter and ice cream.

California

F. A. Gunther, insect toxicologist at the University of California, Riverside, has been named recipient of the Harvey W. Wiley Award of the Association of Official Agricultural Chemists.

The $500 award is presented annually to the scientist who makes an outstanding contribution to the development of methods for the analysis of foods, drugs, cosmetics, feeds, fertilizers, or pesticides, or for use in general analytical chemistry.

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Vitamin D . . . important for absorption and utilization of calcium during growth, pregnancy and lactation . . . is not amply provided by foods listed in the "Guide" . . . unless vitamin D-fortified milk is used in recommended amounts. Vitamin D can be formed in the skin if it is exposed to sunlight or ultraviolet lamp.

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Dr. Gunther has been studying pesticides and their residues for his entire professional career, which began in 1940 at the University of California. He is the author of more than 100 papers covering various aspects of the pesticide field. He also has authored two books.

Dr. Gunther was born in Los Angeles and graduated from the University of Colorado. He received his M.A. and Ph.D. degrees in organic chemistry from the University of California.

Kentucky

November 17-19 have been the dates set for the 1959 annual Dairy Manufacturing Conference at the University of Kentucky.

This conference is sponsored jointly by the Dairy Section of the University of Kentucky and the Dairy Products Association of Kentucky. This is the seventh in the current series of conferences.

Nationally recognized authorities on all types of dairy problems will be on the conference staff. These men will discuss the industry from the source of raw materials to the disposal of waste.

November 17 will be devoted to problems of ice cream, concluding with an ice cream clinic to help Kentucky manufacturers compare their product with those of their competitors.

Market milk will be featured on Wednesday, November 18th and will include discussions on cultured products as well. A cultured product clinic will be featured and a milk scoring contest will be held. The annual banquet held in conjunction with the Tri-City Dairy Technology Society will be held on that day as well.

The rapidly growing cheese industry of the state will have its day on November 19th. For further information concerning this course, please contact A. W. RUDNICK, Dairy Section, University of Kentucky, Lexington, Kentucky.

Louisiana

Louisiana State University has announced the appointment of two new staff members in dairying. They are J. W. ABADIE, instructor in dairying, and J. H. GHOLSON, professor of dairying.

Dr. Gholson, associate professor of extension at the University of Minnesota, received his B.S., M.A., and Ph.D. degrees from the University of Missouri in 1938, 1941, and 1953, respectively. He is a specialist in dairy manufacturing, dairy bacteriology, and dairy chemistry.

Before joining the Louisiana staff, Dr. Gholson served as an instructor and assistant professor of dairy at the University of Missouri, as a county supervisor for the Farm Security Administration, and as a commissioned officer in the U. S. Air Force.

Mr. Abadie received his B.S. and M.S. degrees in 1955 and 1959, respectively, from Louisiana State. He majored in agricultural extension education. Before his present appointment, he was a research assistant in the L.S.U. Dairy Dept.

Pennsylvania

More than 350 persons attended the 17th Annual Dairy Fieldmen's Conference at Pennsylvania State University under the sponsorship of the University and the Pennsylvania Association of Milk Dealers.

The program was devoted to the interests of fieldmen, quality control workers, procurement people, sanitarians and public health personnel. Topics included problems with bulk tanks, cleaning tanks and tankers, the tank truck operator, milking machine problems, materials handling for the dairy herd, barnyard paving, bedding requirements for cows, cow pools, milk conveyor systems in the barn, and many others.

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Off campus speakers included HOWARD RAGSDALE, Abbotts Dairies, Philadelphia; PAUL GIRTON, Girton Mfg. Company; KELLY SAUNDERS, Diversey Corporation; JAMES WHITE and RICHARD GUTHRIE, Cornell University; LIN HUBER, Franklin County, Pa., dairyman; ROBERT DWYER, Sealtest-Reick fieldman, Pittsburgh; FRED GREENLEAF, Lehigh Valley Cooperative, Allentown; and EARL WARNER, Warner's Dairy, Red Lion, Pa.

Virginia

A bulletin, Looking into the Grade A Dairy Industry, has just been published by Virginia Polytechnic Institute. The bulletin contains information on Virginia's Grade A dairy farms. The data were obtained from a survey conducted recently from the dairy farms, milk producers associations, and Virginia milk commission records.

The Virginia Agricultural Extension Service and the Virginia Dept. of Agriculture cooperated in the preparation and writing of the booklet. Individual representatives were G. C. GRAF and M. F. ELLMORE, Dept. of Dairy Science, V.P.I.; G. D. KITE, Dept of Agricultural Engineering, V.P.I.; A. J. ORTEGO, JR., Dept. of Agricultural Economics, V. P. I.; and R. F. HUTCHESON and T. L. STUART, representing the Virginia Department of Agriculture.

This is the first in a series of bulletins to be prepared on a survey of Virginia's dairy industry.

Wyoming

H. S. WILLARD, Professor of Animal Husbandry, is currently in Kabul, Afghanistan, under a two-year contract with the University of Wyoming team of the Foreign Operations Mission. He is engaged in Agricultural and Dairy instruction at Kabul University.

W. R. THOMAS, Assistant Professor of Animal Production, has been granted a leave of absence to work toward the Ph.D. degree at Iowa State University, commencing in June, 1959.

Vacancies on the staff created by Professors WILLARD and THOMAS have been filled by B. W. THARP and M. E. OLDHAM.

BUSINESS AND INDUSTRY NEWS

Borden's News

R. A. GORDON, formerly general manager of Borden's Cities Ice Cream Division at Elgin, Illinois, has been named assistant general manager of the Chicago Ice Cream Region of the Borden Company. R. R. JONES, formerly sales manager at Elgin, becomes manager of the Cities Ice Cream Division. At the same time it was announced by Borden's that J. F. MALONE has been named administrative assistant to the general manager of the Chicago Milk Region.

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Acquisition of Puerto Rico Dairy, Inc., a fluid milk processor and distributor in the capital city of San Juan, has been announced by the Borden Company.

The acquisition is another step in Borden's expanded foreign program. Last February the company acquired Holanda, S.A., an ice cream company, and Mixturas, S.A., a manufacturer of ice cream mixes, both of Mexico City.

Puerto Rico Dairy was established in 1944, and holds milk license "No. 1." It has approximately 120 employees and operates 40 routes. It was purchased from the Lopez family, who continue in managerial positions. The company will continue under its present title, with gradual introduction of the Borden name and its corporate symbol, Elsie the Cow.

Klenzade News

W. F. WALDECK has joined Klenzade Products, Inc., as director of research and laboratories. Dr. Waldeck is well known throughout the chemical industries for his important technical contributions to the advancement of detergent science. He is a graduate of New York University with a B.S. degree in chemical engineering and a Ph.D. in inorganic and physical chemistry. He is a member of the American Chemical Society, Institute of Chemical Engineers, Sigma Xi, Tau Beta Pi, with a distinguished alumnus citation from New York University in 1954.

For over 17 years he was Research Chemist and Director of Laboratories for a prominent detergent chemical corporation where his important technical work substantially contributed to the growth of the company. In his new position with Klenzade, Dr. Waldeck will be in charge of expanded Klenzade research programs devoted to product improvement and the utilization of newer scientific advances in detergent chemistry.

National Dairy Research Center

E. J. HOHHOF, Western Springs, Ill., has been promoted to associate manager in the technical services laboratory of the National Dairy Research Center, Glenview, Ill. Hohhof joined the company in March, 1959, as a senior food technologist. He is a graduate of Northwestern University where he majored in chemistry. He is a member of the American Chemical Society, the Institute of Food Technologists, and the American Oil Chemists Society.

H. W. JACKSON, Mundelein, Ill., has been promoted to group leader in the fundamental laboratory. He joined the company in 1954 as a cheese specialist and has recently been working as a chemist. He is a graduate of the

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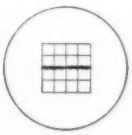
The Farrall Index is described in the Journal of Dairy Science, 24.539 and 26:893-907. The latter, on pages 895-896 gives more detail and a slightly modified procedure. This method is used for research and for quality control.



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University of New Hampshire and earned a Ph.D. degree from the University of Connecticut in 1954.

LOUIS KLEIN, Chicago, has been promoted to senior mechanical designer in the engineering research laboratory. He joined the company in 1954 as a mechanical draftsman. He attended the Armour Institute of Technology.

From the Secretary's Desk

H. F. Judkins

It is indeed a pleasure to present a final summary report of the membership campaign for 1959. The members of the Membership Committee are certainly to be congratulated for their efforts. A few 1959 memberships are still being received; in fact three have come in since this report was prepared. Of course, changes of address are constantly being received which change the count by states.

Now I am looking forward to the 1960 campaign and materials have already been or-

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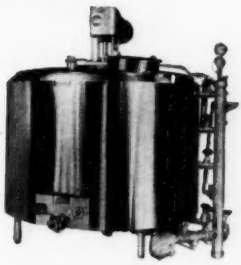

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SOME ARTICLES PUBLISHED IN 1957-58.

"Progress in the Engineering of Dairy Processing" by J. K. Scott.

"Mechanization of Milling, Salting and Hooping in Cheddar Cheese Manufacture" by J. Czulak, N. H. Freeman and H. L. C. Chapman.

"The Stability of Vitamin A in Reconstituted Fortified Non-Fat Milk Solids, Part I—The Effect of Heat; Part II—The Effect of Light"—by J. Conochie and R. A. Wilkinson.

"Modern Packaging of Cheese" by W. J. Park.

"The Effect of Temperature on the Keeping Quality of Butter in Cold Storage" by E. G. Pont and L. F. Gunnis.

"Variations in Lactic Acid Production in Milk" by Barbara P. Keogh.

Obtainable through booksellers, or from the Australian Society of Dairy Technology, P.O. Box 20, Highett, Victoria, Australia. Price $3.50 per annum.

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dered from the Garrard Press to be ready to distribute in October.

New Members		Total Members	
1. N. Carolina	34	1. Illinois	224
2. Illinois	31	2. New York	198
3. Georgia	31	3. Ohio	138
4. New York	25	4. Wisconsin	137
5. Foreign	23	5. California	117
6. Virginia	18	6. Pennsylvania	113
7. Canada	17	7. N. Carolina	103
8. Indiana	15	8. Foreign	102
9. Wisconsin	13	9. Canada	86
10. California	13	10. Michigan	79

Number of states with six to twelve new members	12
Number of states with one to five new members	27
Number of states with no new members	5
Total new members as of July 20, 1959	384
Total number of members as of July 20, 1959	2585

New Affiliates		Total Affiliates	
1. New York	31	1. New York	49
2. California	22	2. Minnesota	46
3. Ohio	18	3. Illinois	42
4. Wisconsin	18	4. Wisconsin	41
5. Minnesota	16	5. Ohio	35
6. Illinois	14	6. California	31
7. Indiana	14	7. Michigan	29
8. Missouri	14	8. Pennsylvania	27
9. N. Carolina	14	9. Missouri	24
10. Mississippi	14	10. Indiana	22

Number of states with six to thirteen new affiliates	19
Number of states with one to five new affiliates	19
Number of states with no new affiliates	6
Total new affiliates as of July 20, 1959	390
Total affiliates as of July 20, 1959	715

American Association for the Advancement of Science
Section O—Agriculture

Symposium for the 1959 Annual Meeting, Chicago, Illinois, December 28-31, 1959

Germ Plasm Resources in Agriculture: Development and Protection

This symposium was organized by R. E. HODGSON of the Animal Husbandry Research Division, U. S. Department of Agriculture, Beltsville, Maryland, and newly elected Vice-President of the American Dairy Science Association—a co-sponsor of this symposium. In developing this program, Dr. Hodgson has been assisted by the leading animal and plant geneticists in the U. S. Department of Agriculture at Beltsville and by H. S. SPRAGUE at Pennsylvania State University. The program follows.

Session I—Origin of Germ Plasm—C. O. ERLANSON, Beltsville, Maryland, Chairman

1. Geographic Origin of Plants Presently Useful to Agriculture—J. R. HARLAN, Stillwater, Oklahoma
2. Origin of Animal Germ Plasm Presently Used in North America—H. H. STONAKER, Fort Collins, Colorado
3. Untapped Sources of Animal Germ Plasm—R. W. PHILLIPS, Washington, D. C.
4. Discussion Leader—H. A. WALLACE, Farvue Farm, South Salem, New York

Session II—Need for and Utilization of Additional Sources of Germ Plasm—H. J. SLOAN, St. Paul, Minnesota, Chairman

1. Horticultural Crops—F. P. CULLINAN, Beltsville, Maryland
2. Field Crops—M. G. WEISS, Beltsville, Maryland
3. Small Farm Animals—A. W. NORDSKOG, Ames, Iowa
4. Large Farm Animals—J. L. LUSH, Ames, Iowa
5. Discusser—H. A. RODENHISER, Washington, D. C.

Session III—Developmental Programs in Crops and Livestock—E. J. WARWICK, Beltsville, Maryland, Chairman

1. Use of Diverse Germ Plasm in Crop Improvement—H. J. GORZ, Madison, Wisconsin
2. Effectiveness of Selection for Animal Improvement—G. DICKERSON, Niles, California
3. Use of Hybrid Vigor in Plant Improvement—G. W. BURTON, Beltsville, Maryland
4. Extent and Usefulness of Hybrid Vigor in Animal Improvement—L. N. HAZEL, Ames, Iowa
5. Performance Testing in Livestock—C. E. TERRILL, E. J. WARWICK, N. D. BAYLEY, W. A. CRAFT and P. B. ZUMBRO, Beltsville, Maryland
6. Discussion—R. E. COMSTOCK, St. Paul, Minnesota

Session IV—New Approaches to Plant and Animal Improvement—A. E. BELL, Lafayette, Indiana, Chairman

1. Contributions of Laboratory Animals to Research in Livestock Improvement—A. B. CHAPMAN, Madison, Wisconsin
2. Immunogenetics and Its Application to Livestock Improvement—M. R. IRWIN, Madison, Wisconsin
3. Using Germ Plasm for New Products—Q. JONES, Beltsville, Maryland, and I. WOLF, Peoria, Illinois

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4. Irradiation and Plant Improvement—R. S. CALDECOTT, St. Paul, Minnesota
5. Possibilities for Genetic Improvement of Useful Insects—R. I. SAILER, Beltsville, Maryland
6. Discussion—W. V. LAMBERT, Lincoln, Nebraska

Session V—Perpetuation and Protection of Breeding Stocks—R. MAGRUDER, Washington, D. C., Chairman

1. Perpetuation and Protection of Germ Plasm as Seeds—E. JAMES, Fort Collins, Colorado
2. Perpetuation and Protection of Germ Plasm as Vegetative Stock—R. E. LARSON, University Park, Pennsylvania
3. Preservation of Breeding Stocks Through Semen Storage—N. L. VANDEMARK, Urbana, Illinois
4. Identification and Elimination of Defects in Animals—F. B. HUTT, Ithaca, New York
5. Discussion—T. C. BYERLY, Washington, D.C.

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THE SURFACE TENSION OF MILK. A REVIEW[1,2]

C. H. WHITNAH
Department of Chemistry, Kansas State University, Manhattan

SUMMARY

Methods of measurement of surface tension applicable to milk have been reviewed. Objectives of surface tension studies included: detection of adulteration; relations of surface tension to the formation of foams or emulsions; to bacterial growth; to the species, ass, buffalo, cow, goat, mare, whale, and woman; to heat treatments; to homogenization; and to developed off-flavors. Milk derivatives studied include: (a) solutions of the proteins casein, β-lactoglobulin, Rowland albumin and globulin, and σ-proteose; (b) wheys, acid, rennet, salt, or ultrafilter; (c) milk acidified with hydrochloric, lactic, or butyric acid; and (d) cream, ice cream, or evaporated milk. Free fatty acids and proteins appear to be the surface-active constituents causing the surface tension of milk or milk products to be lower than that of water.

Brief reviews in 1936 (8), in 1946 (3), and in 1958 (71) have noted a scarcity of data on the surface tension of milk. A cursory survey of the complexities and uncertainties found in surfaces on surface-active materials in pure liquids, and in mixtures containing only two components, might leave one surprised at the abundance rather than the scarcity of studies on such a complex material as milk. Relationships between a solvent and a single surface-active solute have been extensively studied for application to synthetic detergents. Features that might be applied to milk have been reviewed by Moilliet and Collie (49), who also cite many other reviews. Milk fat, some of the milk proteins, and free fatty acids are some surface-active constituents of milk which undoubtedly affect both surface properties (e.g., surface tension) and also bulk properties (e.g., micelle or globule formation) in milk or liquid milk products. Clayton (14) has used milk and modified milk as illustrative material for a general study of many problems in surface activity. Rules have been suggested for relationships between the surface tension and vapor pressure of mixtures (79). These rules have also been applied to other physical properties (e.g., compressibility). It was found they do not always hold, even for binary mixtures (15).

METHODS OF MEASUREMENT

Methods used in measuring the surface tension of milk have been described as static, dynamic, or semidynamic. A static method should yield an equilibrium value, or at least a value that does not change during several times the interval required for measuring. Careful studies on the static surface tension of milk have used capillary rise, the DuNuoy ring, the Wilhelmy plate, or bubble pressure. Methods, using the weight of a falling drop or the number of drops falling from a given volume of milk, have been called dynamic by some workers and semi-

Received for publication June 12, 1959.

[1] Contribution No. 585, Kansas Agricultural Experiment Station.

[2] Reprints available for 90 days after publication of this paper. For prices refer to page 1434 of the August issue of this JOURNAL.

dynamic by others. The shape of a pendant drop may be described as a dynamic method when the time of changing surface composition is long compared to the time of formation of the drop. However, the same uncertainty as in the falling-drop methods arises when the time of measurement is near the time required for formation of the drop. The pendant-drop method apparently has not been used for milk or milk products. The name semidynamic has been used to describe a value which includes to an uncertain degree both the age of the surface and the approach to surface equilibrium. DuNuoy or Wilhelmy methods applied to the surface on a continuously overflowing container have also been called dynamic (10, 11, 14, 17, 30, 31, 33, 34, 35, 49, 51, 62, 64, 71, 73). A critical bibliographical study of the methods of measurement of dynamic surface tensions has recently appeared (19). Drop numbers for aliquots of a sample of skimmilk, when flow times from a stalagmometer were adjusted by changing the air pressure above the sample, decreased from 59.5 at 21 sec. to 52.8 at 374 sec. (46).

Two recent studies of the vibrating jet method have evaluated and refined its use with jets of surface-active solutions (30) and with jets whose surfaces are retarded by friction (31). The method without these refinements has been used in two studies of milk. Both studies agree that the surface tension of milk did not change during the observed age interval of the jet. The first study concluded that the change had not yet begun and that the surface tension of the jet was equal to that of water (42). The second study concluded that the surface tension of the jet was equal to the static value for milk, or that the change was completed during the early part of the first wave (77). Extension of Bohr's method to a fifth approximation in a jet, where the axial ratio $a/b = 2$, changed the calculated surface tension by 0.8%. A table, expressing values of Bohr's ratio n/m in terms of axis ratio a/b, reduced the time needed to calculate a surface tension value by about half. A telephoto camera lens provided a magnified image while the lens was positioned, so that the jet pictures occupied one quadrant of the lens field (75).

A possible explanation of the above conflict is that the earlier workers did not consider differences in density and viscosity between milk and water. The difference in density probably is not significant. The importance of viscosity differences depends on the average diameters and velocities of the jets. These values were not reported in the earlier work. The large decreases of surface tension, in the later study during the interval 0.001 to 0.01 sec. for diluted milk, or for milk from which proteins had been precipitated, seem to justify the second conclusion.

Capillary rise applied first on filter paper, and later much more exactly in glass tubes, has been rated as the most accurate method for measuring static surface tension (73). Obtaining a satisfactory contact angle for a surface-active solution in a capillary tube has been challenged (51, 62). The capillary rise method also has been vigorously defended under particular conditions for milk products (35, 43, 64). Capillary rise values have been found parallel to, but lower than, falling-drop values (46). They have also been found to "compare

with'' (equal?) values using a Wilhelmy plate method or falling drop methods if the drops formed slowly enough (78).

ADULTERATION

Perhaps the simplest objective in studying the surface tension of milk has been to detect adulteration with water or starch (28, 44). Studies with other objectives have observed effects of added water (3, 77) or ionic solutions (43, 56, 64), and of foreign fats (21) or fatty acids (46, 52). Other features of change in surface tension (3, 46, 77) clearly rule out surface tension as a simple test for adulteration.

FOAMING TENDENCY

Milk is one of several systems where tendency to foam and foam stability have been studied in relation to surface tension (49). Falling-drop numbers were early used to measure surface tension and viscosity in relation to the cleansing foam of soap solutions (33). It has been claimed that ". . . it is not low surface tension per se that ensures foam stability but the nature of the adsorbed layer giving rise to the lowering of surface tension." (54). The proteinaceous material eluted by dilute sodium chloride from commercial caseins of high foaming tendency carried the foaming tendency. Small changes in the surface tension of the eluate were found on aging to 6 hr., on boiling, on concentrating at 50° C. and rediluting, and on diluting fivefold. Dilutions of 100- and 1,000-fold produced larger rises in surface tension, and 5,000-fold produced a value slightly higher than water (1). Increased foam stability of aqueous solutions of purified milk proteins with higher concentrations of caseinate, albumin, or lactoglobulin could not be explained by changes in surface tension. The surface tension values were not depressed by concentrations of 0.0001%, but reached minimum values in the range 0.01% to 0.05% (26). The foaming tendency of milk serum, prepared by ultrafiltration of skimmilk, was reduced half by adding 1% skimmilk, without significant change in surface tension (42). At 25° C. or lower, where a phospholipid-protein type foam predominated in simplified systems containing milk constituents, large changes in foaming tendency accompanied only minor changes in surface tension. In some mixtures higher temperatures produced a decrease of 25% or more in surface tension with or without large changes in foaming tendency (60).

EMULSIONS

Emulsion stability and small fat globules are promoted by lowered interfacial tension. The nearly equal lowering of 12.06 and 12.04 dynes/cm for "hard fat" and cocoa fat did not explain differences found in emulsifying power. The lowering of interfacial tension also was very uniform for different skimmilks (47). The surface and interfacial tensions between skimmilk and different fat fractions did not differ sufficiently to be of importance in the theory of butter formation (48). Ice cream mixes containing several emulsifiers and/or stabilizers did not have important differences in surface tension. Stabilizers produced small increases, and emulsifiers slight decreases. Averages were: control 50.02, with stabilizer 53.48, with both stabilizer and emulsifier 48.20 dynes/cm (59).

BACTERIAL GROWTH

Bacterial growth as affected by the surface tension of milk should be considered in relation to older studies on effects of surface tension in other media. In media whose surface tension could be depressed to 32 dynes/cm by castor oil soap, it was found that all pellicle formers ceased to grow at surface tensions less than 45 dynes/cm, and that some anaerobes grew aerobically on media of low surface tension (41). The nature of the depressant, the kind of bacteria, and sometimes the salt concentration of the medium were found to be important factors affecting the relation of growth to surface tension (4). Rancidity development in cold milk accompanied a decrease in surface tension. This decrease has been considered the cause of retarded growth of *Streptococcus lactis* in rancid milk (66). Surface tension depressed with Tween 40 did not retard growth of *S. lactis* in milk. Toxic specific fatty acids, rather than lowering surface tension, were suggested as the cause of the above effect. Oleic acid lowered surface tension but not growth rate, whereas caprylic acid lowered growth rate but not surface tension (16).

SPECIES

Surface tension values for milks of seven species are collected in Table 1. Only those values for cow's milk which were measured along with milk of one

TABLE 1
Surface tension of milks from different species

Ref.	No. & Yr.	Species						
		Ass	Buffalo	Cow	Goat	Mare	Whale	Woman
		(surface tension, dynes/cm)						
40	1911			36.2		37.4		37.4
9	1913		44.0	44.1		44.0		
9	Boiled		46.0	48.4		44.2		
55	1916	56.8		55.2	51.8			50.4
55	Cooled 1 hr. 5° C.	56.2		51.5	50.2			47.4
55	Whey (HCl)	51.5		56.0	57.0			
55	Whey-cooled	51.2		55.8	56.0			
7	1922			46.7				46.1
7	Shaken			58.6				60.4
7	Skimmed			48.2				48.1
36	1941			47.7	48.6			48.5
53	1953						39.1	
5	1953				47.5			
45	1954			48.1	47.5			
13	1955				48.2			

or more other species are included in this table. Some values have been converted from other units to dynes/cm, and some averages have been calculated from individual reported values. The variations between species in lines do not appear larger than the variations in columns, due either to different observers or to known differences in the treatment of the milk. Treatment with ultrasonic vibrations has been reported to lower the surface tension of women's milk but not the milk of cows or goats (36). Increased casein content was found to de-

crease surface tension, whether the casein particles were large, as in mare's milk, or small, as in women's milk (40).

DERIVATIVES

It was early shown that the surface tension of milk increased with decreased concentration of fat or casein caused, respectively, by skimming or acid precipitation. Replacement of the constituent again lowered the surface tension. Effects of udder inflammations, pregnancy, stage of lactation, and feed also were noted (38). Effects of shaking, heating in various ways, and standing or aging of the solution on the surface tension of solutions of casein in water also have been noted (9). Differences in the relation of the surface tension of whole milk to its acid whey, and of each of these to cooling, have been mentioned (Table 1) for milk of the ass, cow, and goat (55).

Acid numbers in the range 13 to 27 had very little effect on the surface tension of skimmilk, although smaller increases of acidity from added HCl or from added or cultured lactic acid increased the surface tension. Surface tensions at several ages after adding rennet to raw and pasteurized skimmilk are shown in Table 2. The return of the surface tension of the whey to the original value of the skimmilk indicates that caseinate phosphate complex is not the material affecting surface tension. Addition of 0.05% of 3% formalin to milk did not change the surface tension either immediately or after 24 hr. (46).

The surface tension of cream at 3° C. decreased with increasing per cent fat and usually with age. Both surface tension and viscosity were involved in whipping properties (18). One liter of water shaken three days with 1 g. casein and a small amount of toluene dissolved 0.021 to 0.024 g. This solution was forced up through a capillary tube, the top wiped off with filter paper, and the solution allowed to reach its level. Heights were measured at intervals from 10 sec. to 2 hr., and were found to fit the equation $\gamma = e^{-kt^{1/2}}$, i.e., the plot of $X = \log t$ vs. $Y = \log \gamma$ was a straight line (34). A 1% solution of casein in $N/100$ (pH 2.46) HCl and in $N/250$ (pH 6.8) NaOH was studied for effects of age (2 min. to 43 hr.) on surface tension. Changes in pH caused a sharp maximum in surface tension at pH 4.62 and another smaller maximum at pH 3.4. Minima were at about pH 3.8 and 8.5. Values using H_3PO_4 were consistently about one dyne lower than when using HCl. The salts, $NaNO_3$, NaCl, and Na_2SO_4, produced nearly equal linear depressions of surface tension in the concentration range 0 to $M/256$. From $M/256$ to $M/4$ Na_2SO_4 produced about twice the rise

TABLE 2

Surface tension of raw and pasteurized skimmilk vs. addition of rennet

	Minutes after adding:						
	Before adding	0	10	30	50	70	Whey
	(surface tension, dynes/cm)						
Raw	49.0	46.5	47.5	48.8	48.8		49.0
Pasteurized	48.5	45.1	46.7	47.5	48.5	48.5	48.5

produced either by $NaNO_3$ or by NaCl. Glucose 0 to $M/16$ was without effect (34). Within the concentration ranges of normal milk, high protein concentration caused low surface tension, whereas fat concentration had no effect. The change with temperature in the surface tension of milk was similar to changes for butter fat or fatty acids suspended in water (37). Although raw unhomogenized creams and pasteurized homogenized creams had lower surface tensions than milk of 3.7% fat, the surface tensions of 30 and 35% creams would extrapolate to exceed those for milk at slightly over 4,000 p.s.i. (71).

Purified β-lactoglobulin was selected as a suitable protein for use in studying effects on surface tension of two buffers at three ionic strengths, concentrations of 0.05, 0.10, 0.25, and 0.50% protein, and surface ages of 1 to 60 min. Bulk ages up to 1 wk. did not modify surface tension. Except as t approaches zero, the relation of surface tension (γ) to time or surface age (t) was $\gamma_t = \gamma_{1\,min.}/t_b$. No systematic difference was found between acetates of Na and Mg as buffers, although the differences were often large. The two forms of β-lactoglobulin differed in slope of the plot $X = \log t$ vs. $Y = \log \gamma$ at most pH values, although at pH 5.0 to 5.8 the two lines coincided. The plot $X = \log \log$ concentration of protein added to one of these buffers vs. $Y = \Delta\gamma$ (surface tension of buffer minus surface tension of solution) was a straight line. It was proposed that the time change of surface tension involved three stages: (a) diffusion to and adsorption of a protein monolayer on the surface; (b) denaturation of this monolayer (age 1 to 60 min., the only range observed); and (c) effects of multilayers of normal or denatured protein micelles (64).

The surface tensions of casein, albumin, and globulin (Rowland separation) dissolved in buffers were found nearly equal to water at concentrations of 0.0001%. Casein and albumin yielded definitely lower tensions at 0.0005% and were nearly independent of concentrations above 0.1% at 56.5 and 56.0 dynes/cm, respectively. The drop of 0.01% globulin solution was similar to that for 0.0005% solutions of casein or albumin. The value for globulin at 0.05 to 0.5% appeared stable at about 58 dynes/cm, but fell to 51 dynes/cm in 1.0% solution (26). Effects of emulsions of butter fat containing added phospholipid have been discussed under foaming (60).

The casein- and albumin- (Rowland) fractions of milk were found to be more important than the globulin fraction in effects of dilution, up to 100-fold, on the surface tension of milk. Dilution effects were studied for: (a) whole milk, (b) skimmilk, (c) dialyzed skimmilk, (d) a material called σ-protose, and sera after precipitation of protein with (e) HCl, (f) rennet, (g) $Zn(OH)_2$, or (h) UAc_3. The σ-proteose contained about 3% of the total N of the milk. Either isolated and redissolved, or partially purified in residual milk sera, it was found to account for "... the marked surface activity of milk freed from its main proteins" (3). The rates of fall in surface tension with surface age were compared for diluted whole milk, undiluted milk from which the casein had been precipitated by NaCl, and from which whey proteins were also later precipitated by HCl. Results are summarized in Table 3 (77).

When evaporated milk was heated to simulate sterilization in cans, surface

TABLE 3

Rate of fall in surface tension of modified milk

	Diluted $1 \rightarrow 10$	NaCl Ppt. removed	HCl Ppt. removed
Age range sec. $\times 10^1$	20–60–100	10–35–70	10–30–70
Rate of fall dynes/cm/sec	1,700 500	3,200 171	5,500 200

tension increased and provided a better measure of coagulation than visual estimates of graininess. Most of this increase in surface tension occurred during the first third of the heating period, with a further small rise near the end of the heating period. Surface tension values ranged from 50.4 to 58.1 dynes/cm (50).

MILK, WHOLE AND SKIM

A. *Effects of heat treatment.*

Cooling milk for 45 min. or longer to less than 10° C. caused an average decrease in surface tension of 2.12 dynes/cm. An average additional drop of 3.64 dynes/cm occurred on standing 12 hr. Rewarming to 20 or 37° C. caused only a small fraction of the reverse change, whereas rewarming to 50° C. restored the original surface tension value. At 45° C., the restoration was about half complete for milk of 3.8% fat, but less for skimmilk of 0.15% fat. The lowering of surface tension on cooling was also much less for such skimmilk. Skimmilk containing 0.50% fat was very similar to whole milk (6, 12, 67).

Values of surface tension by stalagmometer and by the maximum pull on a straight horizontal wire (stirrup method) for averages of three samples of skimmilk, for averages of four samples of whole milk, and for three cream samples of increasing fat content are shown in Table 4.

Effects of temperature of measurement by stirrup method and of per cent fat were studied on skimmilk mixed with increasing amounts of cream from the same milk. Results are shown in Table 5.

Surface tension at 20° C. (by stirrup method) was measured on two samples of whole milk after six heat treatments. The treatments and values are shown in Table 6.

The surface tension of fresh skimmilk was not changed by storage either at room temperature or at 5° C. (46).

At 18° C., and measured 4 to 6 hr. after milking, DuNuoy values of surface tension ranged from 46.6 to 55.3 dynes/cm, with most samples between 49 and 52. Cooling for 1 hr. at 5° C. reduced tension by 0.9 to 1.5 dynes/cm (69).

TABLE 4

Surface tension relative to method and to fat content of milk

	Skim	Whole		Cream	
Per cent fat	.....	.....	13.5	21.0	36.5
Method	*(surface tension dynes/cm)*				
Stalagmometer	52.7	49.6	46.4	45.5	41.7
Stirrup	46.1	39.5	42.9	42.9	43.1
Difference	6.6	10.1	3.5	2.6	—1.4

TABLE 5

Surface tension vs. per cent fat and temperature

Per cent fat	0.04	2–4	10	20	45
Temperature ° C.					
83	38				
20	51.0	46.7	46.2	45.1	44.6
10	51.4	49.0	47.5	47.5	48.5
5	51.6	50.4	49.0	48.7	49.8
1	53				

Surface tension measurements (Wilhelmy at 25° C.) were made on recombined 4% milk to show that the material adsorbed on fat globules differs if the globules are solid or are liquid. The separations were made at 5 or 60° C. with either a hand cream separator or in centrifuged glass tubes. The material was assumed to be adsorbed on solid but not on liquid milk fat globules. For the four combinations containing the material in either the skimmilk or the cream fraction the surface tension of a mixture precooled to 5° C. averaged 2.75 dynes/cm less than for the mixture preheated to 60° C. Corresponding differences with both components enriched or depleted were, respectively, 3.15 and 0.07 dynes/cm (63).

At high temperatures and short times surface tension measurements (DuNuoy at 20° C.) were more sensitive than organoleptic rancidity scores to determine the time-temperature heating needed to inactivate lipolytic enzymes in milk

TABLE 6

Surface tension of whole milk after six heat treatments

Treatment No.[a]	1	2	3	4	5[b]	6
	(surface tension, dynes/cm)					
Trial						
1	47.1	46.8	45.2	44.9	43.6	44.9
2	45.6	.....	44.6	45.2	43.6	44.6

[a] 1. Immediately after milking. 2. Degassed. 3. Storage 3–4 hr. at room temperature. 4. Heating to 60° C. and cooling to 20° C. 5. No. 4 after storage 3–4 hr. at 5° C. 6. No. 5 after heating and cooling as in No. 4.

[b] Only after this treatment was the fat in the solid state when the surface tension was measured.

that had been sensitized by homogenization at 105° F. (40.6° C.). Both tests were made after two days of storage of the heated milk at 400° F. (4.5° C.). The drop in surface tension from the most severe heating measured to the heating which just permitted organoleptic rancidity decreased from 3.0 dynes/cm at 184° F. (84.5° C.) × 0.03 sec. to 0.2 dynes/cm at 145° F. (62.8° C) × 400 sec. Surface tension values after not heating and after holding at 145° F. (62.8° C.) for 200, 300, 400, and 500 sec. were, respectively, 37.9, 43.7, 43.9, 44.4, and 44.5 dynes/cm (32). Average surface tension measurements (DuNuoy) at 60, 81, and 102° F. (15.6, 27.2, and 38.9° C.) were fitted by least squares to parabolas. Values for whole, homogenized, and skimmilk at 60 and 102° F. (15.6 and 38.9° C.), respectively, were 44.5, 47.2, 50.0, and 38.7, 42.0, 46.9 dynes/cm. The increasing values of calculated surface tension for skimmilk at 102° F. (38.9° C.) do not correspond to measured increases in any milk product by any

observer. Two parabolas or even two straight lines, one limited to the range of solid fat and the other to the range of liquid fat, might avoid this difficulty (71).

B. *Effects of homogenization.*

The respective surface tensions (dynes/cm) of skimmilk, either raw and unhomogenized, or pasteurized and homogenized three times at 200 atmospheres with 0, 4, 10, 20, and 30% melted butter oil, were 47.5, 44.2, 42.5, 43.5, 44.8, and 22.6 by a stirrup method, and 51.5, 50.6, 47.1, 47.8, 46.9, and 45.8 by a drop-number method (46). The expanded fat surface in homogenized milk withdrew surface-active materials (protein) from the plasma and raised the surface tension. If lipase was not inactivated by heat, it was activated by homogenization to produce fatty acids which lowered the surface tension. In milk containing less than 5 to 7% fat the latter effect predominated; whereas, with a higher per cent fat, aging was required to overcome the initially predominating former effect. The latter (enzyme) effect was destroyed by preheating the milk to 150° F. (64.6° C.) but not to 140° F. (60.0° C.) (23). Average respective values of surface tension (12 samples by DuNuoy method at 20° C.) after homogenization at

0, 500, 1500, and 2500	p.s.i. were
43.62, 39.61, 38.47, and 39.16	dynes/cm at 90° F. (32.2° C.) and
44.53, 44.97, 45.27, and 45.55	dynes/cm at 145° F. (62.8° C.)

after holding 30 min. (29, 68).

The surface tension of homogenized milk was increased by adding a concentrate of solids-not-fat or by using a pressure of over 3,000 p.s.i. Repeated homogenization or moderate previous addition of cream or water had very little effect, while preheating at 165° F. (73.9° C.) for 30 min. decreased the surface tension (20).

Effects of homogenization have been interpreted as follows: When milk is homogenized, the fat globule membrane becomes dispersed in the plasma and, perhaps, denatured. A new membrane is formed composed of the same or different enzymes, proteins, lipids, etc. proportional to their abundance, mobility, and ability to reduce surface tension. An increase of bound casein in the new membrane may cause the low curd tension in homogenized milk. The new membrane may have lecithin bound outside rather than inside the casein layer (23).

C. *Relations to developed flavors.*

Four aliquots of fresh whole milk, one unmodified, one with $CuSO_4$ added, one with vitamin C added, and one hand homogenized after addition of vitamin C, were diluted with water 10,000 and 100,000 times. Surface tensions of these dilutions (25° C. Wilhelmy) were measured at several surface ages. The induction time, before first decrease in surface tension, varied from a few seconds to 2 hr. These variations were not simply related to either the kind of treatment or the age of the treated sample (76).

Surface tensions (DuNuoy, 20° C. after 4 or 28 hr. at 4° C.) on 852 samples of milk distributed through about 1 yr. are shown in Table 7. Rancidity was

TABLE 7

Rancid flavor vs. surface tension of milk

Flavor class	No. samples	Dynes/cm	Std. dev.	Range
Not rancid	421	46.61	0.95	44.4–50.4
Slightly rancid	193	44.23	1.02	41.8–47.1
Rancid	238	40.74	2.52	32.3–46.1

estimated more conveniently and more exactly by changes in surface tension than by fat acidity determinations (25). Surface tension at age 24 hr. of milk samples from individual cows was used in a study of causes for natural or induced rancidity. Individuality of the cow, stage of lactation, and season of the year each appeared important in some instances, but could not be consistently used to predict the tendency of milk to develop rancidity (26). The rate of fall in surface tension of tenfold dilutions of fresh milk (vibrating jet 20° C.) was greater in those samples which developed oxidized flavor after storage for 3–5 days (74).

D. *Range.*

In addition to the ranges mentioned under previous headings, three other studies of the range of surface tension of milk should be mentioned. It was stated that the surface tension of pure milk has a fixed value of 52 dynes/cm and that dilution or separation had very little effect (39). A range of 48.8 to 55.3 averaging 50.4 dynes/cm (DuNuoy 18–20° C.), with values at 0° C. about 3 dynes/cm lower, was reported (8). Surface tensions of 1,351, 24-hr. samples from collecting stations averaged 55.9 dynes/cm. An unsuccessful effort was made to relate differences in surface tension with suitability of the milk for cheese-making (22).

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ANALYSIS OF MILK PHOSPHOLIPIDS BY CHROMATOGRAPHY AND INFRARED SPECTROPHOTOMETRY

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SUMMARY

Milk pnospholipids were fractionated into the major phospholipid classes by successive elution from a silicic acid column with chloroform, acetone, methanol (20% and 40%) in chloroform, and methanol. Each fraction was analyzed by infrared spectrophotometry as well as by chemical methods. Potassium bromide disk spectra of milk cerebrosides, cephalins, lecithins, and sphingomyelins are presented and compared with pure reference compounds. Concentrations of each class were calculated from specific bands measured either in chloroform or in carbon disulfide solutions. Respective weight percentages of cerebrosides, cephalins, lecithins, and sphingomyelins were: 6, 35, 32, and 24. Evidence is given for the presence of minor amounts of other constituents, including carbohydrate-containing phospholipids, lysolecithins, and unidentified lipids. Advantages and limitations of the method at its present state of development are discussed.

Classical methods for separating and identifying individual phospholipids (3, 10, 23) are often laborious and seldom quantitative, and usually require large amounts of starting materials. An important advance was made in 1952, when Borgström (2) reported that phospholipids could be quantitatively separated from other lipids by adsorption chromatography with silicic acid columns. In his procedure, chloroform was used to elute the nonphospholipids, and methanol to elute all the phospholipids together in a single fraction. Subsequent investigators (5, 6, 13, 15, 17, 22) have shown that further fractionation of the phospholipids is possible by elution with chloroform-methanol mixtures. In general, many small fractions have been obtained and characterized chemically from their hydrolysis products.

Infrared spectrophotometry has been introduced to analyze the intact phospholipid molecules rather than their hydrolysis products. In 1957, Schwarz *et al.* (19) used a combination of infrared and chemical analyses to study numerous small fractions obtained from chromatography of brain lipids and rabbit-skin phospholipids. Nelson and Freeman (14) recently described a simplified method, using infrared spectrometry to determine cephalins, lecithins, and sphingomyelins in relatively small samples of human serum.

With the development of improved methods of lipid separation and analysis, there has been renewed interest in the composition of milk phospholipids; however, all their components have not yet been identified. A number of authors have used differential hydrolysis, either alone (12) or combined with chromatography (1, 4, 7, 18), to estimate the amounts of lecithins, cephalins, and sphingomyelins in various dairy products.

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Rhodes and Lea (18) obtained phospholipids from buttermilk and chromatographed them on a silicic acid column. Cephalin and lecithin fractions were recovered by elution with methanol-chloroform (3:7), and the remainder of the adsorbed choline-containing phospholipids by elution with methanol-chloroform-water (70:25:5). The cephalin and combined choline-containing fractions were examined by chromatographic and chemical methods. In addition to the major constituent phospholipids, minor amounts of lipid-bound inositol and plasmalogens were detected, but cerebrosides were not found. Deutsch *et al.* (4) chromatographed butter phospholipids on a silicic acid column and eluted four fractions with methanol-chloroform (1:4) and methanol. Lecithins and cephalins were identified by paper chromatography, and sphingomyelins by selective saponification. The researchers suggested that cerebrosides were present among the unidentified constituents.

This paper describes a comparatively simple procedure, in which the mixed phospholipids of milk were fractionated into the major phospholipid classes by column chromatography and each fraction analyzed by infrared spectrophotometry as well as by chemical methods. The spectra of milk cerebrosides, cephalins, lecithins, and sphingomyelins are presented and compared with the spectra of pure reference compounds.

EXPERIMENTAL PROCEDURE

Preparation of phospholipid samples. The following reference compounds were used: phrenosin—H. E. Carter; sphingomyelin (spleen)—S. J. Thannhauser; L-α-(dioleoyl)-cephalin (synthetic)—E. Baer; and L-α-(dimyristoyl)-lecithin (synthetic)—La Motte Chemical Products Co.

The method used to isolate mixtures of phospholipids from spray-dried buttermilk has been described in detail (20). Briefly, the lipids were extracted with ethanol, ether, and chloroform, and most of the phospholipids were separated from the other lipids by precipitating them twice with acetone. Acetone-soluble phospholipids were recovered by chromatography with silicic acid. The two phospholipid fractions were combined and washed twice to remove nonlipid contaminants.

Chromatographic separation. For a 1-g. sample, 75 g. of a 2:1 mixture of silicic acid and celite was used in a column (Fischer and Porter Co., Hatboro, Pa.) 2.5 cm. in diameter and 60 cm. long that was fitted with a fritted glass disk and an "Ultramax" Teflon valve. The silicic acid (Mallinekrodt, precipitated) was washed twice with water to remove the smaller particles that remained in suspension. The celite (Johns-Manville, 100-200 mesh) was washed successively with 10 *N* hydrochloric acid, water, and methanol. The mixture of silicic acid and celite was heated at 125° C. for 16 hr. before use and slurred into the column with methanol. A 5-mm. layer of anhydrous sodium sulfate (between filter paper disks) and a perforated porcelain disk were placed on top of the silicic acid–celite column to remove any trace of moisture from the eluants and to protect the top of the adsorbent. Before use, the column was washed successively

with 500-ml. volumes of methanol, acetone, and chloroform. All solvents were reagent grade.

The phospholipid sample was added to the column in 200 ml. of chloroform. Elution was carried out as shown in Table 1. Successive eluants were applied just before the last of the preceding eluant entered the column, and the receiver was changed at the same time. The flow rate varied somewhat at different stages, but averaged about 4 ml. per minute.

Each eluate was reduced in volume in a rotating evaporator under partial vacuum at 50° C. before being transferred into a tared volumetric flask fitted with a S/T stopper. After the remainder of the solvent was evaporated with a stream of nitrogen, the flask was reweighed. Then each flask was filled to its calibration mark with chloroform and flushed with nitrogen. To prevent evaporation, the stopper and top of each flask were covered with Saran film. All samples were stored at 0–5° C. and analyzed within a few days of preparation.

Chemical methods of analysis. Lipid phosphorus was determined as previously described (21), and total nitrogen by the micro method of Ma and Zuazaga (11). The anthrone (16) and ninhydrin reactions (9), respectively, were used as semiquantitative tests for reducing sugars and amino nitrogen. All chemical data reported are averages of at least two determinations.

Infrared measurements. Potassium bromide disks were prepared as follows: An aliquot containing 0.8–2.1 mg. of phospholipid was evaporated in a mortar under a heat lamp with a stream of nitrogen, 400 mg. of potassium bromide (Harshaw, infrared quality) was ground thoroughly with the sample, and the mixture was transferred to an evacuable ½-in. steel die. The die was sealed by applying slight pressure on the plunger with a Carver press, and was then evacuated with a vacuum pump. The pressure exerted on the die was gradually increased to 17,000 lb., which was held for 15 min. The disks were placed in a vacuum desiccator and their spectra were subsequently recorded in the range of 2.5 μ to 15 μ with a Perkin-Elmer Model 137 Infracord spectrophotometer.

Spectra recorded from potassium bromide disks have advantages for qualitative investigations of phospholipids, because they are unobscured by solvent absorption, but the disks are somewhat cloudy for some samples, making quantitative estimations difficult. For this reason, the authors prefer to base quantitative estimations on bands measured in solution.

Infrared absorption spectra of the samples in solution were recorded from 2 μ to 11 μ with a Baird Associates Model B spectrophotometer equipped with a sodium chloride prism. These spectra were obtained from either chloroform or carbon disulfide solutions (depending on solubility) containing 6.8–7.1 mg/ml of sample. The absorption cell used had an optical path length of 0.9 mm.

Absorbance A at each specified absorption maximum was calculated as $A = \log_{10} \frac{T_o}{T_s}$, where T_o is transmittance of the solvent and T_s is transmittance of the sample solution. The amounts of cephalins, lecithins, and sphingomyelins were calculated from available calibration data (14).

TABLE 1

Chemical analyses of fractions from chromatography of milk phospholipids

Fraction	Eluant	Eluant volume	Fraction weight	Lipid phosphorus		Total nitrogen		Amino nitrogen	Reducing sugar	Principal components
		(ml.)	*(mg.)*	*(%)*	*(mg.)*	*(%)*	*(mg.)*			
I	Chloroform	400	84	0.04	0.0	0.20	0.2		−	Nonphospholipids
II	Acetone	500	71	0.34	0.2	1.90	1.4		+++	Cerebrosides
IIIa	20% Methanol in chloroform	100	62	3.05	1.9	1.91	1.2	++	+	Cephalins
IIIb	20% Methanol in chloroform	200	206	3.92	8.1	1.95	4.0	+++	−	Cephalins
IIIc	20% Methanol in chloroform	200	30	4.06	1.2	2.25	0.7	+++	−	Cephalins
IVa	40% Methanol in chloroform	100	42	3.84	1.6	2.48	1.0	++	+	Cephalins
IVb	40% Methanol in chloroform	300	145	4.10	6.0	2.01	2.9	+	−	Lecithins
IVc	40% Methanol in chloroform	600	284	3.86	11.0	2.41	6.8	−	-	Lecithins and sphingomyelins
Va	Methanol	100	85	4.00	3.4	3.47	3.0	−	−	Sphingomyelins
Vb	Methanol	400	51	3.92	2.0	3.28	1.7	−	−	Sphingomyelins
Total recovered			1,060		35.4		22.8			
Total applied			1,003	3.72	37.2	2.12	21.2			

RESULTS AND DISCUSSION

Infrared spectra of reference phospholipids. Potassium bromide disk spectra of phrenosin, L-α-(dioleoyl)-cephalin, L-α-(dimyristoyl)-lecithin, and sphingomyelin are shown (Figure 1). A portion of the spectrum of cephalin in carbon disulfide solution is included in Figure 1-B. Both the phrenosin and sphingo-

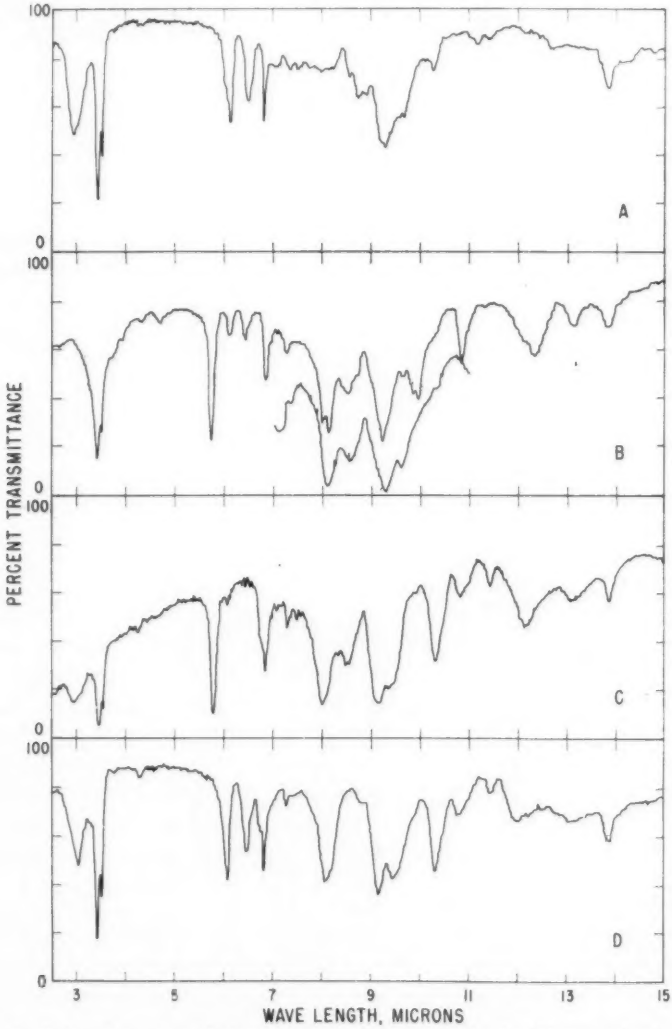

FIG. 1. Infrared spectra of phospholipid reference samples in 400-mg. potassium bromide disks. *A*. Phrenosin, 0.8 mg. *B*. L-α-(dioleoyl)-cephalin: Upper curve—0.9 mg. in KBr, lower curve—10 mg/ml in carbon disulfide. *C*. L-α-(dimyristoyl)-lecithin, 1.5 mg. *D*. Sphingomyelin, 0.8 mg.

myelin spectra correspond to those found by Schwarz *et al.* (19). Sphingolipids, such as sphingomyelins, phrenosins, and other cerebrosides, can be distinguished from other lipids by the strong absorption bands at 6.1 μ and 6.5 μ that are characteristic of monosubstituted amides (-CONH-). (Note that weak bands of uncertain origin appear at these positions in the spectrum of cephalin also.) The sphingomyelin spectrum differs from the phrenosin spectrum, in that the former has a strong band at 8.1 μ, a doublet at about 9.1–9.4 μ, and a stronger band at 10.3 μ.

The spectra of both cephalin and lecithin have, at 5.8 μ, a strong ester carbonyl band that is absent in sphingolipid spectra. Lecithins can be distinguished from cephalins by a strong band at 10.3 μ that is absent in cephalins. The disk spectrum of L-α-(dioleoyl)-cephalin in Figure 1 shows weak bands at about 6.1 μ and 6.4 μ, some absorption at about 9.6–10 μ, and a band at 10.8 μ. Schwarz *et al.* also observed in cephalins, weak absorption at 6.1 μ and a stronger band at 9.8 μ. However, the bands in the 9.8 μ and 10.8 μ regions must be associated with the physical state of the sample, because they do not appear in the solution spectrum of the reference cephalin (Figure 1-B). The spectrum of the reference lecithin corresponds to that reported by Schwarz *et al.* except that, in our curve, the band at about 7.3 μ is much weaker and there is less indication at 6.1 μ for the presence of small amounts of water in the sample.

Analysis of milk phospholipid fractions. During this investigation, five different samples of milk phospholipids (20) were analyzed by silicic acid chromatography and infrared spectrophotometry, but the results for only one sample are presented. These are representative of the earlier work and are more complete.

Weights and chemical analyses of the ten fractions obtained by chromatographic separation of mixed phospholipids from buttermilk are listed (Table 1). The total weight recovered indicates that the eluting solvent was not completely removed from some of the fractions before they were weighed. However, considering the numerous possibilities of error, the recoveries of both lipid phosphorus and total nitrogen were satisfactory. The chemical data indicate that the order of elution was cerebrosides, cephalins, lecithins, and sphingomyelins.

Each fraction was further characterized by infrared spectra and absorbance measurements at specific wave lengths. Potassium bromide disk spectra of Fractions II to Vb are presented (Figures 2, 3, 4, and 6). The composition of each fraction is discussed in the order of elution.

Fraction I. Essentially no phospholipids were eluted by chloroform. The infrared spectrum of this sample did not reveal any significant amounts of cholesterol, glycerides, or fatty acids, but indicated the presence of phthalate and, possibly, carotenoids. When an aliquot of this fraction was dissolved in iso-octane and examined in the ultraviolet region, an absorption peak was found at 275 mμ. Di-*n*-butyl phthalate absorbed strongly at the same wave length. Fraction I probably consisted mostly of phthalate plasticizer that had been present as a contaminant in the original crude phospholipid sample. This fraction was not included in subsequent calculations of the amounts of each phospholipid class.

Fraction II—(Figure 2). The spectrum for this sample, which was eluted

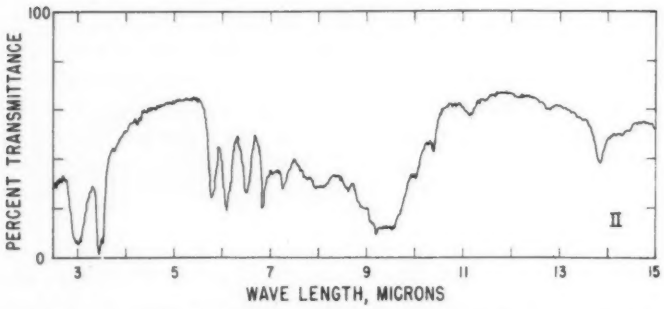

FIG. 2. Infrared spectrum of Fraction II (mostly cerebrosides) from chromatography of milk phospholipids, 2.1 mg. in 400 mg. potassium bromide.

with acetone, resembles in most respects the spectrum of phrenosin (Figure 1-A) and exhibits the characteristic amide absorption bands at 6.1 μ and 6.5 μ. Absorption in the 9–9.5 μ region is attributed to C=O bonds in the sugar part of the cerebrosides.

Unfortunately, calibration data are not available for the 6.1 μ band of a pure cerebroside. However, when the absorption coefficient of sphingomyelin was used, the calculated cerebroside concentration was about 80%. The amount found by chemical analysis was 96%, but less confidence can be placed in this result, because anthrone is not specific for cerebrosides.

The carbonyl ester band at 5.8 μ indicates the presence of cephalins in this fraction. But if cephalins have an average phosphorus content of about 4.0%, this fraction could contain only about 7% cephalins. The concentration of cephalins was 35–40% when calculated from the 5.8 μ band of Fraction II dissolved in chloroform, but excessive absorption at 5.8 μ could be attributed to the presence of small amounts of nonphospholipid esters not completely eluted by chloroform.

In view of the above considerations, and the probable errors involved (14), the following composition for Fraction II was tentatively accepted: cerebrosides 80%, cephalins 10%, unidentified lipids 10%.

Rhodes and Lea (18) found no cerebrosides in the choline-containing phospholipids of milk, but their report did not indicate that they tested their cephalin fraction for reducing sugars. Since, in our work as well as in that of Schwarz *et al.* and Deutsch *et al.*, cerebrosides were eluted before cephalins, it seems possible that the cephalin fraction of Rhodes and Lea may have contained some cerebrosides.

Fractions IIIa, IIIb, and IIIc—(Figure 3). Most of the cephalins present in the original mixture of milk phospholipids were eluted in these fractions with 20% methanol in chloroform. The term cephalins includes both phosphatidyl ethanolamines and phosphatidyl serines, since they are presumably both eluted by this solvent and are virtually indistinguishable from one another by their infrared spectra.

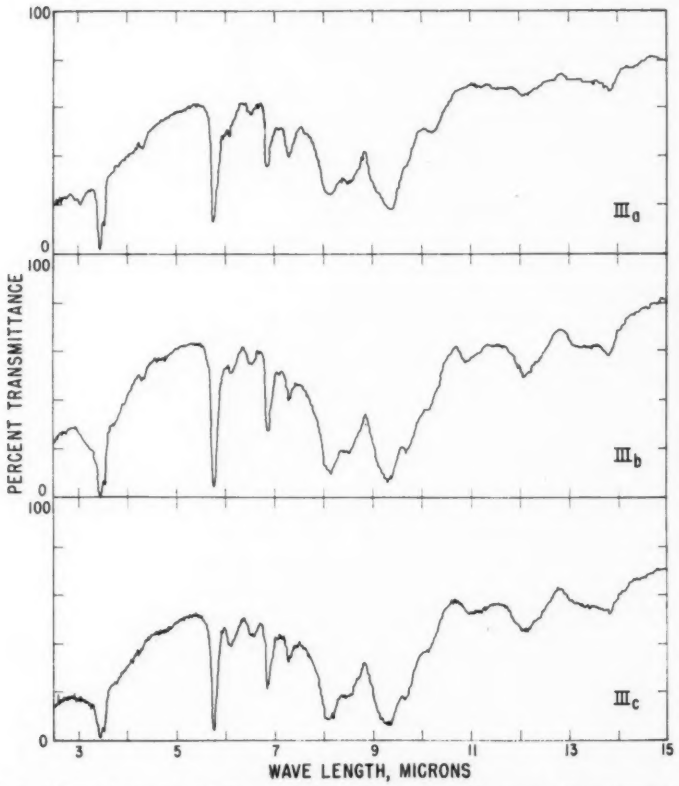

FIG. 3. Infrared spectra of Fractions IIIa, IIIb, and IIIc from chromatography of milk phospholipids in 400-mg. potassium bromide disks. *IIIa*. Cephalins and cerebrosides mixture, 2.1 mg. *IIIb*. Cephalins, 1.2 mg. *IIIc*. cephalins, 1.8 mg.

The disk spectra for the three samples are similar and correspond in most regions to those of the reference cephalin. They are also similar to the spectra for L-α-(dimyristoyl)-cephalin and rabbit skin cephalin (19). The absence of a 10.3 μ band shows that no lecithins or sphingolipids were present in Fractions IIIb and IIIc.

The concentration of cephalins in Fraction IIIa was 98% when calculated from the 5.8 μ band of its chloroform solution, but only around 80% on the basis of phosphorus content. Therefore, an average value of 90% cephalins was accepted. Since the spectrum shows minor absorption at 10.3 μ and the sample gave a positive anthrone reaction, the remaining 10% was probably cerebrosides.

Fraction IIIb was essentially all cephalins on the basis of its absorptivity at 5.8 μ in carbon disulfide. This is confirmed by the percentages of lipid phosphorus and total nitrogen, the strongly positive test for amino nitrogen, and the negative test for cerebrosides.

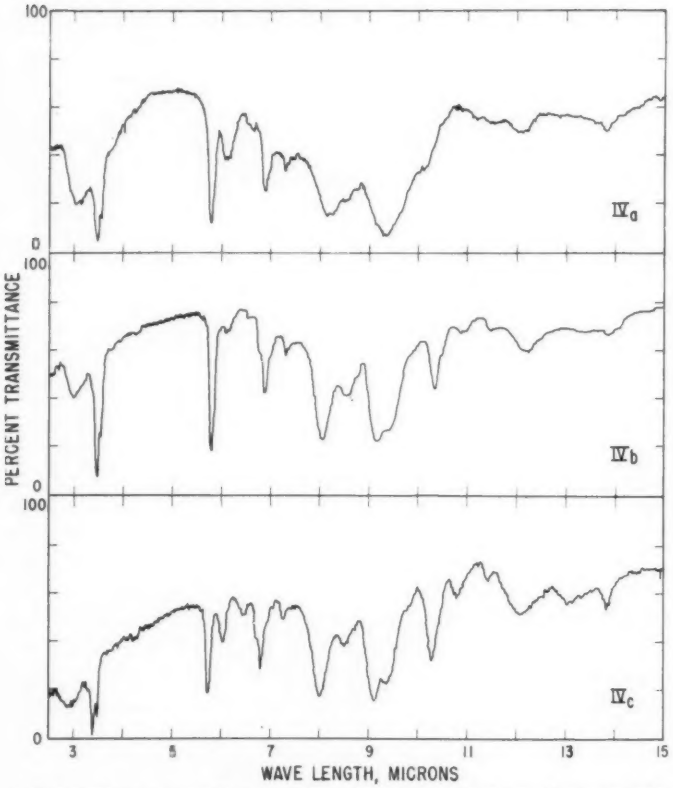

FIG. 4. Infrared spectra of Fractions IVa, IVb, and IVc from chromatography of milk phospholipids, in 400-mg. potassium bromide disks. *IVa.* Mostly cephalins, 2.1 mg. *IVb.* Lecithins, 1.0 mg. *IVc.* Lecithin and sphingomyelin mixture, 1.1 mg.

Fraction IIIc was also calculated to be all cephalins, although the total nitrogen percentage is somewhat higher than the theoretical percentage for cephalins.

Fractions IVa, IVb, and IVc—(Figure 4). The remainder of the cephalins, all the lecithins, and part of the sphingomyelins were eluted in these fractions with 40% methanol in chloroform.

The spectrum of Fraction IVa resembles those of the reference cephalin (Figure 1-B) and of L-α-(dimyristoyl)-cephalin (19), except that its ratio of absorption at 9.3 μ to 8.1 μ is greater. Absence of a band at 10.3 μ shows that there were no lecithins or sphingomyelins present. On the basis of absorption measured at 5.8 μ in carbon disulfide, the concentration of cephalins was calculated as 90%. However, Fraction IVa contained 2.48% nitrogen and gave a positive sugar reaction. Therefore, both the infrared and chemical data indicate that the remaining unidentified 10% of this fraction included sugar-containing lipids—possibly some type of cerebroside or phosphoinositide. Hanahan *et al.*

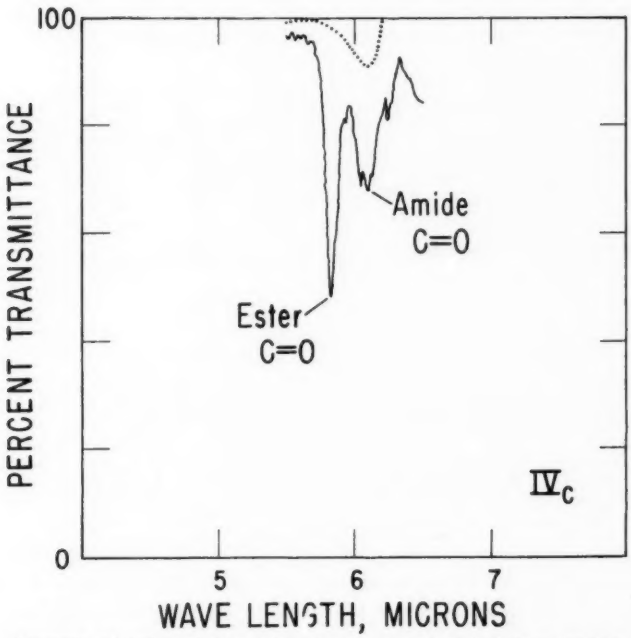

FIG. 5. Example of infrared absorption bands used in calculating lecithin and sphingomyelin in mixed fractions. (Curve shown is for Fraction IVc, 5.68 mg/ml in chloroform.) Dotted line is solvent transmittance.

(6) reported that inositol-containing phospholipids were eluted from silicic acid with 40% methanol in chloroform, and Rhodes and Lea (18) found inositol in a milk phospholipid fraction eluted with 30% methanol in chloroform.

The spectrum for Fraction IVb corresponds very closely to that of the reference lecithin (Figure 1-C). The total amount of lecithins present was 100% and 94%, respectively, when calculated from the absorptivities at 9.15 μ and 10.3 μ. Since this fraction also gave a weakly positive amino nitrogen test and a negative sugar test, a composition of 95% lecithins and 5% cephalins was accepted.

Fraction IVc was a mixture of lecithins and sphingomyelins. This is shown by the strong band at 10.3 μ common to both phospholipids, the amide group bands at 6.1 μ and 6.5 μ found in sphingolipids, and the ester carbonyl band at 5.8 μ attributed to the lecithins present. The concentrations of each component were calculated from the absorbances at 5.8 μ and 6.1 μ, measured in chloroform (Figure 5), using the following simultaneous equations:

$$C_{\text{Lecithins}} = k_1 A_{5.8} + k_2 A_{6.1},$$
$$C_{\text{Sphingomyelins}} = k_3 A_{5.8} + k_4 A_{6.1}.$$

Values for the constants k_1, k_2, k_3, and k_4 were calculated from calibration data (14). Fraction IVc contained 60% lecithins and 40% sphingomyelins. On the

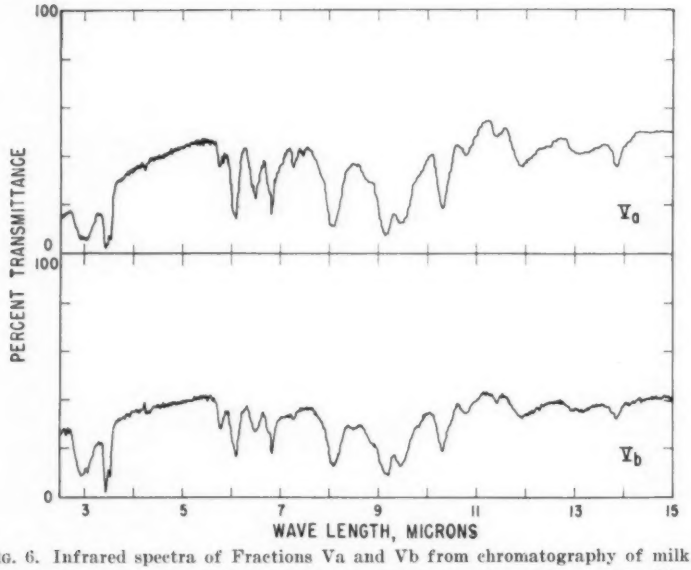

FIG. 6. Infrared spectra of Fractions Va and Vb from chromatography of milk phospholipids, in 400-mg. potassium bromide disks. *Va.* Sphingomyelins, 1.7 mg. *Vb.* Mostly sphingomyelins, 1.0 mg.

basis of absorption measured at 10.3 μ, these two classes of phospholipids accounted for all of the fraction.

Fractions Va and Vb—(Figure 6). These last two fractions were eluted with methanol, and consisted mostly of sphingomyelins. Their spectra correspond with that of the reference sphingomyelin (Figure 1-D), except for the presence of minor carbonyl ester bands near 5.8 μ. The composition of each fraction was calculated, as above, from the absorbances at 5.8 μ, 6.1 μ, and 10.3 μ measured in chloroform.

Fraction Va contained 95% sphingomyelins and 5% lecithins, and the weight calculated at 10.3 μ accounted for all of the sample.

Fraction Vb contained 85% sphingomyelins and 15% lecithins when calculated from the equations. However, the total percentage of phospholipid was 110% from the absorptivity at 10.3 μ. The presence of lysolecithins, which should be eluted after sphingomyelins (15), could account for both the presence of the ester band at 5.8 μ and the increased absorptivity at 10.3 μ. There is no spectroscopic characteristic by which lysolecithin could be distinguished from lecithin in such a mixture. Since it is considered probable that lysolecithins could be present, a composition of 85% sphingomyelins, 10% lysolecithins, and 5% lecithins was tentatively accepted for this fraction.

Composition of milk phospholipids. The proportions of the major phospholipid classes in mixed milk phospholipids were calculated from the estimated compositions of the above fractions, and are compared with data recently reported

TABLE 2

Comparison of major phospholipid classes found in milk by various investigators

Phospholipid class	Present study		Baliga and Basu (1)[a]	McDowell (12)[a]	Rhodes and Lea (18)	Koops (7)	Deutsch *et al.* (4)
	(wt. %)	*(moles %)*[a]	*(moles %)*				
Lecithins	32	31	29	28	33	30	33
Cephalins	35	37	47[b]	43	39	40	38
Sphingomyelins	24	23	24	29	19	25	23
Cerebrosides	6	6	....	....	Not found	Not found	Found

[a] Assuming following average molecular weights: lecithins, 806; cephalins, 744; sphingomyelins, 831; cerebrosides, 826.
[b] By difference.

by others (Table 2). The percentages of lecithins, cephalins, and sphingomyelins found in the present study agree quite closely with those of Rhodes and Lea (18), Koops (7), and Deutsch *et al.* (4). The appreciable amount of cerebrosides found is consistent with the earlier work of Kurtz and Holm (8).

It is concluded that a combination of chromatography and infrared spectrophotometry is very useful for studying milk phospholipids. Chromatographic fractionations can be followed and the principal components readily determined from the spectra. Accuracy is influenced by variations in the extinction coefficients of various lecithins etc., and by the composition of the fractions examined, but is probably equal to that attained by microchemical analysis (14, 19). Only comparatively small samples are necessary for infrared spectrophotometry, and they may be recovered if desired. Isolation and quantitative analysis of constituents occurring in minor amounts, such as lysophospholipids, inositol-containing phospholipids, and plasmalogens, would require both a more elaborate fractionation procedure than that used in the present study and highly purified reference compounds that are not yet readily available.

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STUDIES ON CASEIN. II. THE CARBOHYDRATE MOIETY OF CASEIN

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SUMMARY

The carbohydrate in casein has been estimated by hydrolysis of the protein with a mixture of dilute hydrochloric acid and a cation exchange resin, passage of the hydrolysate through a cation exchange column to remove interfering substances, and the measurement of the colour produced by the reaction of the liberated sugar with an anthrone reagent. The sugar contents of whole casein (Warner), alcohol-extracted casein, α-casein, β-casein, and γ-casein determined in this manner were approximately 3.8, 1.8, 1.5, 0.8, and 0.9 mg/g, respectively.

The individual sugars in the casein preparations were determined quantitatively by filter paper chromatography. Whole casein contained galactose (1.84 mg/g), glucose (1.11 mg/g), and mannose (0.53 mg/g). Alcohol-extracted whole casein contained galactose (1.34 mg/g) and mannose (0.56 mg/g) and α-casein also contained galactose (0.75 mg/g) and mannose (0.48 mg/g). β-casein contained only galactose (0.52 mg/g) and γ-casein contained only galactose (0.48 mg/g).

Sorensen and Haugaard (20) applied the orcinol reaction to the determination of carbohydrate groups in proteins. From the time-curve of the orcinol reaction, they concluded that Hammersten casein contained 0.31% galactose. Sorensen (19) suggested later that the carbohydrate component of casein was a mixture rather than a single substance. This was confirmed by Mesamune and Maki (14), who detected mannose, galactose, and glucosamine in acid hydrolysis of casein by the use of partition chromatography on paper.

Koiw and Gronwall (10) developed a method for staining protein-bound carbohydrate after electrophoresis of serum in filter paper. Their method involved the periodic acid–Schiff technique developed by McManus (16) and by Hotchkiss (7).

Glegg *et al.* (4) pointed out that the hydroxyamino acids, serine and threonine, at the end of the protein chain, and hydroxylysine at any position in the chain, would give rise to a periodic acid–Schiff reaction. However, the terminal amino acids of α- and β-casein have been shown to be restricted to lysine and arginine (17), and it is highly unlikely that casein contains hydroxylysine. It is probable, therefore, that the staining of casein by the periodic acid–Schiff technique is due to the presence of carbohydrate.

MacRae and Baker (12, 13) separated the α-, β-, and γ-components of the casein complex by filter paper electrophoresis and determined the positions of the protein bands by staining with Azocarmine B. Duplicate strips of the paper were stained for carbohydrate according to the method of Koiw and Gronwall (10). They observed staining only at the position of the α-component. In another experiment, in which equal amounts of purified α-, β-, and γ-casein were applied to the paper, the proteins subjected to electrophoresis, and then stained for carbohydrate, staining was observed only at the position of the α-casein band.

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This paper deals with (a) the development of a method for the estimation of protein-bound carbohydate and the application of the method to the determination of the carbohydrate content of casein and (b) the quantitative estimation of the individual sugars in casein by paper chromatography.

MATERIALS

Whole casein was prepared from a composite sample of milk (Macdonald College herd) according to the method of Warner (21). The casein was thoroughly washed with water and dried with alcohol and ether (Preparation No. 1).

Alcohol-extracted casein was prepared by extraction of whole casein (Preparation No. 1) with 95% ethanol in a Soxhlet extractor for 96 hr.

α- and β-casein were prepared from whole casein (Preparation No. 1) by the method of Hipp *et al.* (6) and γ-casein was prepared by the method of Hipp *et al.* (5).

EXPERIMENTAL METHODS

Determination of sugar by the anthrone reaction. Preliminary experiments. It was essential at the outset to find a method for the hydrolysis of the protein which would liberate the sugars from the protein, and at the same time would not lead to sugar destruction.

Casein was hydrolyzed with hydrochloric acid (4 *N*) for periods of time ranging from 1 to 8 hr. The separate hydrolysates were treated with the anthrone reagent (3) before and after the amino acids and amino sugars had been removed from the hydrolysates by means of Dowex 50 (1). The absorbances of the various solutions were measured at wave lengths ranging between 540 and 680 mμ by means of a Beckman Spectrophotometer (Beckman Model DU). Figure 1 shows typical curves obtained with the various solutions [A—hydrochloric acid (4 *N*) hydrolysate before treatment with resin; B—water eluate of Dowex 50 column through which the hydrochloric acid (4 *N*) hydrolysate passed; C—acid eluate of Dowex 50 column through which the hydrochloric acid (4 *N*) hydrolysate had passed]. It will be observed that the characteristic peak at 620 mμ which is given by glucose was not given by the hydrolysate, either before or after treatment with cation exchange resin.

Adopted procedure. Casein (100 mg.) and 5 ml. of a cation exchange resin suspension (one part Dowex 50, 200-400 mesh, in two parts of 0.05 *N* hydrochloric acid) were placed in a Pyrex test tube (13 by 100 mm.). The tube was sealed and then heated in a rotary oven at 100° C. for 16 hr. At the end of the heating period the tube was cooled and opened. The contents of the tube were placed on the top of a Dowex 50 column (10 by 30 mm.) which was in the hydrogen form. The eluate was collected and the column was washed with 10 ml. of distilled water. The eluates were combined and labelled "water eluate." The column was then washed with hydrochloric acid (10 ml., 2 *N*) followed by distilled water (2 ml.). These eluates were combined and labelled "acid eluate." Traces of amino compounds which might be contained in the water eluate were removed by passage of the solution again through the column. The column was

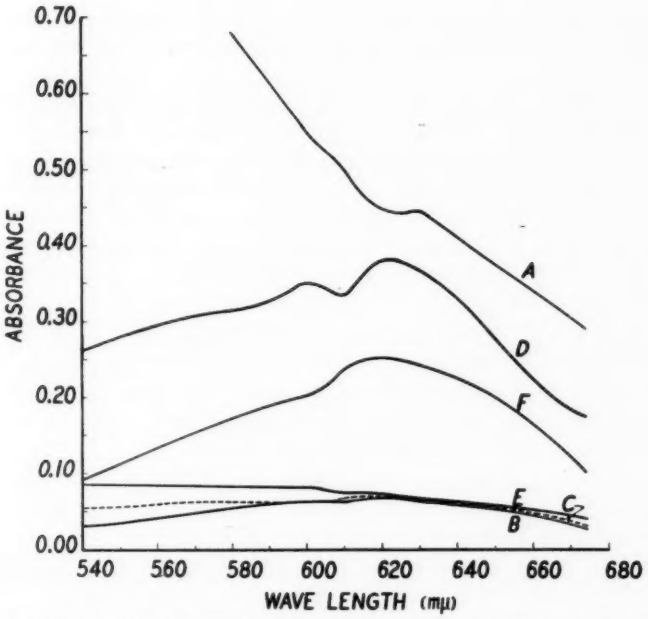

FIG. 1. The effects of methods of hydrolysis and resin treatment on the absorbance of casein hydrolysates after treatment with anthrone reagent.

A—Hydrochloric acid (4 *N*) hydrolysate before treatment with Dowex 50; B—hydrochloric acid (4 *N*) hydrolysate after treatment with Dowex 50; C—acid eluate of Dowex 50 column through which the hydrochloric acid (4 *N*) hydrolysate had passed; D—Solution S_1 (water eluate from resin hydrolysis); E—Solution S_2 (acid eluate from resin hydrolysis); F—glucose standard.

then washed with water (8 ml.) and the eluates were combined and the volume adjusted at 25 ml. This solution (S_1) was assumed to contain the sugar which was to be determined. The column was then washed with hydrochloric acid (8 ml., 2 *N*) and this eluate was combined with the "acid eluate" and the volume adjusted at 25 ml. This solution (S_2) was assumed to contain the amino sugars.

The sugar was determined by the method described by Fairbairn (3). Two, 2-ml. portions of the water eluate (S_1) were each treated with 10 ml. of the anthrone reagent. The colour was developed by heating the mixture in a boiling water bath for 12 min. The solutions were cooled and their absorbances were measured at 620 mμ by means of an Evelyn colorimeter. The interval between the end of the heating period and the time of measurement of absorbances was 20 min.

A standard curve was prepared by plotting the absorbances (620 mμ) of standard glucose solutions (5, 10, 20, 30, 40 μg/ml) which had been treated with the anthrone reagent, against the concentrations (μg/ml) of the standard solutions. The sugar concentration in the water eluates and, hence, the percentage sugar

(reported as glucose) in the casein sample, was obtained by referring the average of the absorbances of the duplicate samples of the unknown solution to the standard curve.

A sample of casein was hydrolyzed with the resin mixture, the hydrolysate passed through the resin column, and the water eluate (S_1) treated with the anthrone reagent. The resin column was then eluted with hydrochloric acid (2 *N*) and the eluate (S_2) treated with the anthrone reagent. The absorbances of the two solutions (S_1, S_2) were measured at various wave lengths (540-650 mμ) by means of a Beckman Spectrophotometer (Model DU). Figure 1 shows that the characteristic peak at 620 mμ obtained with the glucose solution and the water eluate was not obtained with the acid eluate (S_2).

Effects of period of hydrolysis. Samples of casein were treated with the Dowex 50 hydrochloric acid mixture as outlined previously, for periods ranging from 6 to 36 hr., and the sugars were determined on the resulting hydrolysates. The results for hydrolytic periods of 6, 10, 16, 24, and 36 hr. were 0.7, 0.67, 0.80, 0.60, and 0.63 mg. of sugar per gram of casein, respectively.

Determination of hexosamine. Ten milliliters of the combined acid eluates (S_2), obtained in the procedure for the determination of sugar by the anthrone reaction, were evaporated to dryness in a vacuum desiccator to remove hydrochloric acid. The residue was dissolved in 5 ml. of distilled water. Hexosamine was determined (1) in triplicate on 0.5-ml. portions of this solution.

Chromatographic methods. Identification of sugars. The method of McFarren *et al.* (15) was employed for the separation of galactose, glucose, and mannose by filter paper chromatography. The sugars were stained on the paper by use of aniline hydrogen phthalate (18).

Quantitative determination of the sugars. The same solvent system and staining agent were used as for the qualitative identification of the sugars. The procedure for the application of the standards and unknown solutions to the paper was essentially that described by Baker and Khan (2). However, instead of employing equal quantities of different dilutions of the standard as in the previous work, different quantities (4, 6, 11, 14, 18 μl.) of a standard solution containing 1.67 μg/ml of each sugar (galactose, glucose, mannose) were applied to each paper. The solution was applied in 1-2-μl. quantities and the spots were dried between application. Four spots containing the unknown were applied to each paper. Twenty microliters of the appropriate hydrolysates was used for each spot in the analysis of whole casein and 30 μl. was used for each spot in the analyses of the α-, β-, and γ-casein.

The procedure for measurement of the maximum colour density of the spots, preparation of standard curves, and evaluation of the results have been described previously (9). No filter was employed in the measurement of the maximum colour density.

RESULTS AND DISCUSSION

The sugar content of casein. Whole casein and also the α-, β-, and γ-fractions of the casein complex were analyzed for their sugar contents. The analyses were

performed on duplicate hydrolysates (Series 1, Series 2) and each result reported in Table 1 represents the average of duplicate determinations on each hydrolysate.

The hexosamine content of cow casein reported by Johansson and Svennerholm (8) was 1.8 mg/g and by Anastassiadis and Common (1), 1.66 mg/g. The total carbohydrate (hexosamine, hexose, sialic acid) of cow casein reported by Johansson and Svennerholm (8) was 8.0 mg/g.

TABLE 1

Sugar and hexosamine content of casein[a]

Sample	Sugar (by anthrone)		Hexosamine	
	Series 1	Series 2	Series 1	Series 2
	(mg/g)			
Whole casein (Warner)	3.81	3.88	2.24	2.16
Whole casein (alcohol-extracted)	1.77	1.76	1.64	1.68
α-casein	1.55	1.48	2.12	2.11
β-casein	0.75	0.79	1.15	1.10
γ-casein	0.87	0.86	2.37	2.48

[a] Based on 15% nitrogen.

Identification of the sugars in casein. Duplicate samples of whole casein (150 mg.) were heated with the resin suspensions (5 ml.), and the resulting hydrolysates were treated with the resin column by the adopted procedure described above. The duplicate water eluates (S_1) were combined and then were evaporated to dryness in vacuo. The residue was dissolved in 0.3 ml. of distilled water and 30 μl. of the resulting solution (S_3) was applied to the paper in one spot. The chromatogram was prepared as described in the section on Methods. The results showed that either the main sugar constituent was an unknown sugar with an Rf value less than that given by the galactose standard, or that it was galactose which was giving an Rf value less than that given by the standard. Two other smaller spots were observed on the paper, one with a slightly lower Rf value than the mannose standard and another with a slightly lower Rf value than the glucose standard.

In another experiment, a mixture of 10 μl. of the standard mixture (5 μg/ml of each sugar—galactose, glucose, mannose) and 30 μl. of solution S_3 was applied to the paper and the chromatogram was prepared. Three spots with Rf values approximately the same as those obtained with S_3 alone were observed. This experiment showed (a) that the presence of the hydrolysate reduced the Rf values of the sugars and (b) that the three spots which were obtained with S_3 corresponded to galactose, glucose, and mannose.

The interfering substances in S_3 were removed from the solution by passage of Solution S_3 through a column (13 by 76 mm.) of Permutit S in the carbonate form. The column was washed with water after the passage of S_3 through the column and the combined eluates and wash water evaporated to dryness in vacuo. The residue was dissolved in 300 μl. of distilled water. Chromatograms were prepared with this solution (S_4), and three spots having Rf values identical to those given by galactose, glucose, and mannose standards were obtained.

Solutions (S_4) were prepared using alcohol-washed whole casein, α-, β-, and γ-casein. The chromatograms obtained with these solutions and also with the solution (S_4) prepared from whole casein are shown in Figure 2.

FIG. 2. Identification of sugars in casein by filter paper chromatography.

Quantitative estimation of the individual sugars in casein. The sugars in S_4 were determined quantitatively by filter paper chromatography. Table 2 shows the results obtained with whole casein, alcohol-extracted whole casein, α-, β-, and γ-casein. Each sugar was determined on duplicate hydrolysates and each result represents the average of eight determinations.

Sugar recovery. Duplicate samples (0.3 ml.) of a sugar solution containing 1.66 μg/ml of each sugar (galactose, glucose, mannose) were treated in exactly the same manner as was the casein in the preparation of S_4. The sugars in the resulting solution were determined by the filter paper chromatography method and the following recoveries were realized: galactose, 78.0%; glucose, 74.4%; mannose, 75.8%.

When electropherograms of whole casein were examined by the periodic acid–Schiff technique, a positive reaction was observed in the α-casein zone but not elsewhere. When electropherograms of α, β-, or γ-casein (prepared by precipitation methods) were examined in the same way, only α-casein gave a positive reaction (13). These observations were confirmed in the course of the present study. By themselves, these observations might lead to the supposition that the carbohydrate of casein is confined to the α-fraction. However, the work now reported shows clearly that sugars can be detected in hydrolysates of all three fractions when the hydrolysis is performed with a resin–dilute acid mixture.

Leblond *et al.* (11) have suggested that freely reactive 1,2-glycol groups in carbohydrate-protein complexes may be partially responsible for the positive periodic acid–Schiff test given by some of these materials. The results of the present study suggest that the carbohydrate-protein complex in β- and γ-caseins may not contain freely reactive 1,2-glycol groups.

Filter paper chromatography was employed for the quantitative and qualitative analysis of the sugars present in whole casein (Warner), alcohol-extracted casein (Warner), α-casein, β-casein, and γ-casein. The whole casein preparation, which had been washed with water and then with alcohol and with ether to remove moisture, contained galactose, glucose, and mannose in the ratio of approximately 3.5:2.0:1. The nitrogen content was 15.05% (dry matter basis). It was considered possible that this preparation might contain an appreciable

TABLE 2

Sugar content of whole casein and of casein fractions[a]

	Series 1			Series 2		
	Galactose	Glucose	Mannose	Galactose	Glucose	Mannose
	(mg/g)					
Whole casein (Warner)	1.85(0.095,[b] 5.1[c])	1.11(0.028,[b] 2.5[c])	0.53(0.020,[b] 3.8[c])	1.83(0.080,[b] 4.6[c])	1.10(0.028,[b] 2.55[c])	0.53(0.014,[b] 2.64[c])
Whole casein (alcohol-extracted)	1.34(0.025, 1.87)	0.00	0.56(0.028, 5.0)	1.34(0.025, 1.9)	0.00	0.56(0.020, 3.57)
α-Casein	0.74(0.045, 6.1)	0.00	0.47(0.028, 6.0)	0.75(0.078, 10.4)	0.00	0.49(0.035, 7.1)
β-Casein	0.51(0.040, 7.8)	0.00	0.00	0.53(0.032, 6.1)	0.00	0.00
γ-Casein	0.47(0.032, 6.8)	0.00	0.00	0.49(0.025, 5.1)	0.00	0.00

[a] Based on 15% nitrogen.
[b] Standard deviation.
[c] Coefficient of variation.

amount of residual lactose. Accordingly, the preparation was subjected to Soxhlet extraction with 95% alcohol for 96 hr. The casein was then dried. The nitrogen content was 15.42% (dry matter basis). The extraction had reduced the galactose content by approximately 28%, but the mannose content was practically unchanged. Glucose, moreover, could no longer be detected by filter paper chromatography.

The simultaneous reduction of the galactose and glucose contents of whole casein by alcohol extraction could be explained by the removal of lactose contamination. However, if lactose were the only contaminating sugar removed by alcohol extraction, then the reduction in galactose content might be expected to be approximately equal to the reduction in glucose content. The fact that approximately twice as much glucose as galactose was removed by alcohol extraction suggests that glucose must have been present in the casein preparation in some other form as well as lactose.

It will be observed, from the data reported in Tables 1 and 2, that the sugar contents of α-, β-, and γ-casein do not account for the carbohydrate of the whole casein. This may point to a loss of carbohydrate-containing material during the preparation of the subfractions of the whole casein. The yield of α-, β-, and γ-casein in no instant accounted for 100% of the original casein, and it is possible that the material lost in preparation may have a high carbohydrate content. However, this point can be settled only by further research.

It is of interest to note that β- and γ-caseins contain only galactose, while α-casein contains galactose and mannose. It remains to be seen whether mannose is responsible for the fact that, of the casein fractions, only α-casein gives a positive periodic acid–Schiff test.

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A RAPID METHOD OF DETERMINING DISPERSIBILITY OF NONFAT DRY MILK SOLIDS

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SUMMARY

A mechanical method of determining dispersibility of nonfat dry milk using a Hobart Model 3C Kitchen Aid Mixer is described. A 5-sec. stirring time is used which gives better differentiation of grades than can be obtained by previously described procedures using longer stirring times. The mixture is screened and the amount of solids dispersed is determined by a hydrometric procedure developed for this purpose. The hydrometric procedure used was demonstrated to be as accurate as gravimetric determination of solids by oven drying. The approximate percentages of various types of nonfat dry milks dispersed using this procedure are: instant, 80%; ordinary spray-dried, 37%; and roller-dried, 0%. The differences in dispersibility observed between various brands of instant milks are large enough to be significant for grading purposes. The procedure, besides readily separating nonfat dry milk into dispersibility classes, has the feature of being very fast, the whole procedure requiring only 10 min.

The ease with which dry milk samples disperse in water is an important factor in evaluating their quality. Several methods (2, 10, 11) have been described for the objective determination of dispersibility, none of which has gained general acceptance.

Stone *et al.* (11) have described an objective method employing mechanical stirring with a Hobart Model 3C Kitchen Aid Mixer, followed by screening and determination of the amount of material which has gone into solution. Methods based on this procedure have recently been studied collaboratively under the supervision of the American Dry Milk Institute (2). The collaborative study indicated that some improvement in the methods is desirable.

One of the major problems in dispersibility measurements is the rapid and accurate determination of the dissolved solids. Two methods of determining total solids in milk are described by the A.O.A.C. (1). The first of these involves drying a weighed sample of milk to constant weight at 100° in an oven, whereas the second, described as an approximate method, uses the Quevenne lactometer readings and the per cent fat to calculate total solids. A number of firms now manufacture special equipment for rapid moisture determination by drying.

Considerable work has been done with determination of solids by the use of lactometers, and highly refined equations have been developed for the calculation of total solids from the specific gravity and the per cent fat (3, 4, 8, 12). Reinart and Nesbitt (7) compared some of these procedures with the Mojonnier gravimetric method and found they agreed fairly well. Madden *et al.* (5) also compared a variety of lactometric procedures with gravimetric results and obtained fair agreement.

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Michle *et al.* (6) compared the Mojonnier method, Cenco moisture balance, and the Karl Fischer titration, and concluded that the Mojonnier method was much superior. The A.D.M.I. collaborative study (2) on dispersibility methods included a comparison of the Mojonnier and the A.O.A.C. Method I. The Mojonnier method was found to give less satisfactory results in some cases.

The present paper describes a rapid method of determining dispersibility. It is based on the method of Stone *et al.* (11), with some important modifications which contribute to its simplicity and precision.

A. *Apparatus*

Mixer—Hobart Model 3C Kitchen Aid, equipped with standard agitator and automatic (mercury tilting) switch for starting agitator when the agitator is in the bowl.[1]

Sieves—8 in., 60-mesh and 100-mesh, together with collecting pan.

Graduated cylinders—250 ml.

Balance—Torsion or any balance which will weigh accurately to 0.1 g.

Hydrometer—Range 1.000–1.070 calibrated to 0.0005 subdivisions (Fisher).

Flasks, pipets, weighing dishes, stirring rods.

Timer—One which may be set to turn off stirring after a predetermined time.

Thermometer.

B. *Procedure*

The sieves are arranged with the 60-mesh on top of the 100-mesh and the collecting pan below. The stirrer is set at Speed 2 (approximately 77 r.p.m.) and hooked in the circuit with the timer, which is set to turn off after 5 sec.: 235 ml. of water at room temperature (20–30° C.) is poured into the mixing bowl, 35 g. of the milk powder to be tested is weighed out and carefully poured on the water in the mixing bowl. The agitator is immediately lowered into the mixing bowl, turned on and allowed to run 5 sec., at which time the clock will automatically stop the stirring.

The agitator is then removed, the liquid swirled once in the bowl, then poured into the sieves. The sieves may be shaken gently to speed filtration, which should be complete in 1 min. The liquid in the pan is then poured with the aid of a stirring rod into a 250-ml. graduated cylinder and water is added to the 250-ml. mark. The liquid is mixed thoroughly by pouring into a beaker and back again. Foam on the surface is drawn off carefully with a pipet attached to an aspirator. It is usually advisable to allow the sample to stand 5 min., to allow all foam to rise to the surface.

The hydrometer is placed in the liquid and allowed to bob up and down to come to equilibrium. The hydrometer is read to the graduation nearest the top of the raised area around the stem. The temperature of the milk is also recorded.

[1] The mention of specific instruments or trade names is made for the purpose of identification and does not imply any endorsement by the U. S. Government.

C. *Calculation of results*

The hydrometer should be checked for accuracy with water. If necessary, a correction may be added or subtracted to give the true density of the solution. The hydrometer reading (H) gives the specific gravity of the sample relative to water at 4° C., and must be divided by the density of water at the temperature of the sample to give the specific gravity (G) relative to water at this temperature.

To calculate the per cent solids, the formula for calculating total solids used in the A.O.A.C. Method II (lactometric) (1) was modified to

$$0.25\ (1{,}000)\ (G - 1) + 0.3 = \%\ \text{solids}. \tag{1}$$

The amount of fat in nonfat dry milk is small and relatively constant and so may be neglected when this equation is used.

The hydrometer reading (H) times 250 gives the total weight of the solution. The number of grams of the powder which dissolved may be calculated by the formula

$$\text{Grams dissolved} = \frac{250 \quad (\text{H}) \quad (\%\ \text{solids})}{100}. \tag{2}$$

The sample of dry milk may be assumed to contain 3% moisture unless the true moisture content is known. The actual amount of solids in a 35.0-g. sample then is 34.0 g. Formula (3):

$$\%\ \text{dissolved} = \frac{\text{weight dissolved} \times 100}{34.0} \tag{3}$$

or, combining Formulas (2) and (3),

$$\%\ \text{dissolved} = \frac{250 \quad (\text{H}) \quad (\%\ \text{solids})}{34.0} \tag{4}$$

Illustration

A sample calculation to illustrate the use of the above formulas follows:

A. Calibration of hydrometer:

H_2O at 23° C.	
Hydrometer reading	0.9990
Density H_2O at 23°	0.9976
Approximate correction	−0.0014

B. Calculation of per cent dispersed (sample):

Hydrometer reading	1.0370
Correction (from above)	−0.0014
Corrected hydrometer reading (H)	1.0356
Temperature	24°
Density of water at 24°	0.9973 g/ml

Specific gravity (G) $= \frac{1.0356}{0.9973}$

$= 1.0385$

From Formula (1)

% solids $= 0.25\ (1{,}000)\ (G-1) + 0.3$

$= 0.25\ (38.5) + 0.3$

$= 9.9\%$

From Formula (4)

% dispersed $= \frac{250\ \ (H)\ \ (\%\ \text{solids})}{34.0}$

$= \frac{250\ (1.0356)\ (9.9)}{34.0}$

$= 75.5\%$

RESULTS AND DISCUSSION

In preliminary experiments with a procedure based on that of Stone *et al.* (10), it was observed that a much better spread between samples was obtained if the stirring time was shortened from 10 to 5 sec. It appears that 5 sec. is insufficient time to control accurately. However, in practice this was no problem and good precision was invariably obtained, even when the stirring time was controlled manually. The temperature of the water ranged from 20–30° C., which had no noticeable effect on the rate of dispersion in this temperature range.

The greater spread between samples of various dispersibilities obtained by the shorter stirring time means that less precision is required to obtain meaningful results.

A specific gravity method for determining solids was adopted to speed up the procedure. Commercial lactometers are designed for use at lower temperatures than were convenient for our work; therefore, an ordinary hydrometer was used.

To calculate the per cent of dissolved solids, the formula from the A.O.A.C. Method II (lactometric) (1) was tested. The formula is:

$$0.25L + 1.2\ (\%\ \text{fat}) = \%\ \text{solids} \qquad (5)$$

The term (L) corresponds to 1,000 (G−1). One per cent fat was assumed to be present in the dry powder in calculating results using this equation. This will give a fat concentration near 0.1% in the reconstituted milk.

The accuracy of this method was checked by completely dissolving known amounts of different nonfat dry milk samples in water and comparing the actual amount dissolved with the results obtained from the formula. The percentages of solids calculated from Equation (5) were a little lower than would be expected from the amount dissolved.

Modification to:

$$\%\ \text{solids} = 0.25\ (1{,}000)\ (G-1) + 0.3 \qquad (1)$$

gave a good fit with the experimental data. Fat content of nonfat dry milk is assumed to be small and relatively constant and is allowed for in the constant.

Table 1 gives a comparison of the results obtained when a known amount of dry milk was dissolved in water and the resulting solution was analyzed for total solids by the specific gravity and the A.O.A.C. oven methods (1). We found it necessary to increase the drying time to 6–8 hr. by the oven method, to get constant weight and results comparable to the actual amount dissolved. In calculating the amount dissolved, allowance was made for the small amount of moisture in the dry powder. Moisture was determined by drying to constant weight at 100° C.; or, in the case of some instant samples, it was assumed to be 3%, as specified on the label.

TABLE 1

A comparison of the A.O.A.C. oven method with the specific gravity method of determining per cent solids in reconstituted nonfat milk

Sample	Known solids	Specific Gravity Method	A.O.A.C. Method
		(%)	
1	5.83	5.8	5.8
4	7.60	7.6	7.9
5	7.60	7.6	7.5
4	9.35	9.3	9.2
1	9.48	9.4	9.4
2	9.45	9.3	9.7
4	10.05	10.1	10.1
3	11.21	11.1	11.1
6	11.3	11.3	11.2
6	11.3	11.3	11.2
4	12.6	12.6	12.8
5	12.6	12.6	12.4

Our data show that the specific gravity method described is entirely satisfactory and invariably gives close agreement with the actual amount of dry milk dissolved. Somewhat better precision and accuracy were obtained from specific gravity measurements than from the A.O.A.C. oven method. In all but one case, the specific gravity method gave results agreeing within $\pm$ 0.1% of the actual amount dissolved. This is within the limit of error in reading the hydrometer, and is comparable to the accuracy obtained with other lactometric procedures (3, 4, 8, 12).

Table 2 shows the dispersibilities of various dry milk samples, including five different brands of instant milk powders, two ordinary spray-dried powders, and two roller-dried powders. Instant powders were about 80% dispersible by this procedure, whereas the ordinary spray-dried powders were only about 37% dispersible. Roller-dried samples were practically nondispersible by this procedure. The large differences in dispersibility observed allow the three types of dry milk to be readily differentiated. The reproducibility of duplicate determinations was sufficient to allow significant differences to be observed between individual samples of each type.

Many instant milk powders which appeared to disperse completely with no visible lumps formed a granular sludge in the bottom of the mixing bowl, which

TABLE 2
Dispersibility measurements

Sample	First determination	Second determination
	(%)	
Instant dry milks[a]		
Brand A	78.6	79.5
Brand B	80.5	79.0
Brand C	85.0	85.5
Brand D	79.0	77.7
Brand E	83.3	82.1
Ordinary spray-dried milks		
Sample 1	36.7	34.7
Sample 2	39.0	38.8
Roller-dried milks		
Sample 3	Approx. 0.0	Approx. 0.0
Sample 4	Approx. 0.0	Approx. 0.0

[a] Samples A–E were different brands of commercial instant dry milks purchased at a supermarket.

was collected on the screens. The amount of this undispersed material collected on the screens depends on the mesh of the screens and, therefore, if results are to be compared with those reported here, the size and mesh of screens specified must be rigidly followed. Some samples which gave visible lumps after stirring were completely free of this sludge. Therefore, visible lumps alone are not a completely reliable criterion of dispersibility.

Instant milk powders become lumpy and appear to lose their desirable dispersibility properties if not stored in tightly sealed containers or in a desiccator. This occurs in hot, humid, summer weather. This effect was not observed with ordinary spray-dried powders, which did not deteriorate noticeably under the same conditions. More study is needed on the stability of instant milk powders under various conditions.

These procedures are applicable only to nonfat dry milk. Problems of determining both total solids by specific gravity and dispersibility are much simplified, since the physical state of the fat in the milk is of no concern. The effects of fat are discussed by Heinemann *et al.* (3) and by Watson (12) for lactometric determination of solids, and by Sinnamon *et al.* (9) and Stone *et al.* (10) for dispersibility determinations.

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The ordinary spray-dried and roller-dried samples used in this study were obtained from the Chicago USDA, Agricultural Marketing Service Dairy Inspection Laboratory.

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GROWTH OF CERTAIN LIPOLYTIC MICROORGANISMS AT 4 C. AND THEIR INFLUENCE ON FREE FAT ACIDITY AND FLAVOR OF PASTEURIZED MILK

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SUMMARY

Twenty-five pure cultures of lipolytic microorganisms sometimes found in market-milk were inoculated into samples of high-quality pasteurized whole milk subsequently held at 4 ± 1° C. for a period of 12 days. At four-day intervals during the hold period the samples were examined for microbial content, free fat acidity, and developed flavors. Eighteen cultures grew reasonably well and brought about increases in free fat acidity. Seventeen of these produced a rancid flavor sometimes accompanied and/or followed by bitterness, whereas one caused the milk to be criticized as sour and unclean.

It has been known for many years that microbial lipolysis may contribute to the degradation of milk fat. But with the apparently increasing importance of psychrophilic microorganisms as a detriment to market milk quality, this phenomenon may be of more concern to the dairy industry today than in the past.

Palmer (10) and Rice and Markley (11) seem to have been among the first investigators to appreciate fully this type of lipolysis, as they were careful to incorporate preventatives in their experiments on the natural lipolytic activity in milk. More recent workers have published affirmative information. Anderson and Hardenbergh (2) reported the spoilage of refrigerated cream by an organism resembling *Achromobacter* or *Alcaligenes*. During this spoilage the only biochemical change worthy of note was the hydrolysis of fats, but there developed a bitter flavor accompanied by an irritating after-effect. Hammer and Collins (7) found that unsalted, raw cream butter held for as few as seven days at 6° C. frequently developed rancidity and contained large numbers of lipolytic bacteria. Davis (5) believed that bitterness in milk held at a low temperature might well be the result of butterfat degradation rather than the formation of "peptones." Babel (3) reported that the acidity of milk fat increased slowly in milk held at 4.4° C. and abrupt increases were generally accompanied by a marked increase in numbers of psychrophiles. Thomas (12) concluded that *Pseudomonas fluorescens* and *Pseudomonas fragi*, both known to grow very well at 5° C., may be the most common organisms responsible for rancidity occurring in butter. Such reports suggest a need for further information concerning the detrimental effect that lipolytic microorganisms may have on the quality of market milk held at refrigeration temperatures.

This study was made to determine the extent of fat hydrolysis and flavor development accompanying the growth in refrigerated, pasteurized whole milk of certain lipolytic microorganisms sometimes found in market milk.

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MATERIALS AND METHODS

Twenty-five pure cultures were used in this study. Twenty of these, No. 1 through 20, were isolated in the laboratory from samples of market milk held in refrigerated storage for various periods of time after purchase on the local market. These cultures were characterized and keyed to genus only, according to Breed *et al.* (4). Five cultures were obtained from the American Type Culture Collection (ATCC), Washington, D. C. Nineteen of the 20 laboratory isolates were distributed among three genera of bacteria, whereas Culture No. 16 was a yeast. The genera with their included cultures were *Pseudomonas,* No. 1 through 12; *Alcaligenes,* No. 13, 14, and 15; and *Achromobacter,* No. 17 through 20. The ATCC cultures were No. 21, *Alcaligenes viscolactis* (9036), No. 22, *Brevibacterium lipolyticum* (11367), No. 23, *Flavobacterium suaveolens* (958), No. 24, *Pseudomonas fluorescens* (11251), and No. 25, *Pseudomonas fragi* (4973). Each of the 25 cultures demonstrated the ability to hydrolyze butterfat within 15 days at 5° C., ten days at 10° C., and two days at 21° C. This was determined by streaking a loopful of a 24-hr. broth culture on Milk-Protein Hydrolysate (M-PH) agar containing 1% butterfat dyed with Nile Blue A, according to the method described by Knaysi (8), and incubating the streaked plates until positive results were obtained.

Stock cultures were maintained on M-PH agar slants in screw-cap culture tubes held at 4 ± 1° C. These were rejuvenated at monthly intervals by passing them through a 24-hr. incubation period in Tryptose Phosphate Agar (TPA) broth (1) at 21° C., then to fresh slants incubated 24 hr. at 21° C. before replacing them in 4 ± 1° C. storage. Working cultures were maintained in TPA broth in cotton-plugged culture tubes. These were transferred at seven- to ten-day intervals and held at 4 ± 1° C. after 24 hr. incubation at 21° C. Milk sample inocula were prepared by transferring a loopful of working culture to a tube of TPA broth and incubating it for 24 hr. at 21° C. A loopful of the 24-hr. culture was then transferred to 10 ml. of sterile skimmilk in a screw-cap culture tube which was held six days at 4 ± 1° C. before being used to inoculate a milk sample to be studied.

The milk samples used in this study were commercially pasteurized whole milk obtained from the University Creamery. All milk used originated in the same mixed-breed herd of cows. Fat content was standardized to between 3.5 and 4.0% before pasteurization. The average initial free fat acidity (FFA) as determined by the method of Frankel and Tarassuk (6) for all samples was 3.8. No milk was used having a flavor criticism on the day obtained, other than the cooked flavor. Within 15 min. after the end of the 30-min. hold period, quantities of milk of approximately one liter were aseptically removed from the pasteurizing vat and placed in sterile two-liter Erlenmeyer flasks packed in flake ice. These were quickly transferred to a room held at 4 to 6° C. The Erlenmeyer flasks were closed with rubber stoppers encased in butter parchment to prevent the adsorption of flavors from the stopper or the external atmosphere. Each series consisted of six samples of milk, one as a control and each of the other five inoculated with a pure culture of the lipolytic organism. The results of ten such series, including ten controls and duplicates each of 25 pure cultures, are reported.

The samples after cooling to 5 to 10° C. were inoculated with an amount of a six-day skimmilk culture estimated to give a Standard Plate Count (SPC) of not more than 30,000 per milliliter. However, with one trial of two cultures this number was exceeded. After inoculation, the samples were violently agitated and placed in refrigerated storage at $4 \pm 1°$ C. Daily each sample was hand agitated until all visible traces of cream adhering to the flask were obliterated.

Within 30 min. after inoculation and on the fourth, eighth, and 12th day of refrigerated storage approximately 150 ml. were aseptically removed from each sample to a chilled, sterile 250-ml. Erlenmeyer flask which was then closed with a parchment-encased cork. These 150-ml. portions were removed immediately after hand-agitating the sample with 50 vertical strokes of about 2 ft. The portions were plated for both SPC and psychrophilic plate count (PPC) while still at storage temperature. The plate counts were made according to *Standard Methods for the Examination of Dairy Products* (1), using M-PH agar. Plates for SPC were incubated at 32° C. and those for PPC were incubated at 5° C. for seven days. After plating, the remainder of each 150-ml. portion was warmed to $20 \pm 1°$ C. in a water bath. After sufficient hand-agitation to effect fat dispersion, duplicate 10-ml. volumes were pipetted into 60-ml. centrifuge tubes for determination of free fat acidity (6). The remaining milk was then examined organoleptically by an experienced milk judge, assisted at times by one or two others. All flavor criticisms as made by the judge were recorded, with no attempt to assign numerical scores to the samples.

RESULTS AND DISCUSSION

As indicated by the plate counts recorded in Table 1, 18 of the 25 cultures studied grew reasonably well in pasteurized whole milk held at $4 \pm 1°$ C. Even though all cultures used in this study grew and hydrolyzed butterfat on an agar plate at 5° C., four in the genus *Achromobacter*, No. 17 through 20; one *Alcaligenes*, No. 13; *Alcaligenes viscolactis* (9036), No. 21; and *Flavobacterium suaveolens* (958), No. 23 made little or no growth in the pasteurized milk during the 12-day period. Possibly this was due to the relative size of the inoculum used on the agar plate and in the pasteurized milk sample.

As indicated in Table 1, Cultures 2, 10, and 11 produced high counts on the psychrophilic plates, but these cultures were unable to develop countable colonies at 32° C. as shown by their Standard Plate Counts. Also, with many of the other cultures higher counts were obtained at 5 than at 32° C. Nelson and Baker (9) found that many bacteria from refrigerated milk failed to develop countable colonies at 32° C.

The very low average SPC maintained by the ten control samples during the hold period is of some interest. Also, the apparent absence of psychrophilic microorganisms in the control samples aseptically removed from the pasteurizing vat agrees with the reports of several workers, as reviewed by Thomas (12), that efficient pasteurization destroys those psychrophilic types commonly found in milk. During preliminary trials of this study several attempts were made to utilize commercially pasteurized milk available to this Station from different

TABLE 1

Standard plate counts (SPC) and psychrophilic plate counts (PPC) on samples of pasteurized whole milk inoculated with pure cultures of lipolytic microorganisms and held 12 days at 4± 1° C. Each (except for the control) is the average of two trials per culture

	Days of storage							
	0[a]		4		8		12	
Culture	SPC	PPC	SPC	PPC	SPC	PPC	SPC	PPC
	(plate counts/ml)							
Control[b]	320.0	<10	330.0	<10	320.0	<10	310.0	<10
1	4.9T[c]	47.0T	42.0M[d]	85.0M	82.0M	310.0M	180.0M	340.0M
2	390.0	990.0T	1.9T	33.0M	3.7T	110.0M	2.3T	170.0M
3	6.4T	34.0T	21.0M	59.0M	57.0M	160.0M	100.0M	260.0M
4	2.7T	44.0T	6.1M	78.0M	56.0M	150.0M	130.0M	150.0M
5	6.7T	6.3T	6.5M	13.0M	450.0M	490.0M	490.0M	490.0M
6	9.8T	35.0T	1.2M	16.0M	32.0M	130.0M	150.0M	290.0M
7	15.0T	18.0T	82.0M	14.0M	230.0M	310.0M	600.0M	360.0M
8	7.8T	9.8T	260.0T	530.0T	24.0M	71.0M	37.0M	88.0M
9	21.0T	45.0T	2.1M	9.1M	28.0M	23.0M	190.0M	650.0M
10	360.0	240.0T	650.0	1.4M	550.0	180.0M	580.0	360.0M
11	320.0	230.0T	320.0	1.9M	680.0	160.0M	590.0	570.0M
12	8.8T	9.0T	240.0T	550.0T	48.0M	160.0M	350.0M	130.0M
13	9.3T	700.0	12.0T	230.0	33.0T	790.0	140.0T	850.0
14	83.0T	70.0T	710.0T	900.0T	58.0M	68.0M	460.0M	420.0M
15	56.0T	24.0T	480.0T	390.0T	150.0M	130.0M	380.0M	340.0M
16	2.0T	1.5T	29.0T	31.0T	1.7M	960.0T	31.0M	6.4M
17	15.0T	17.0T	670.0	560.0	2.0T	630.0	290.0	80.0
18	4.3T	2.3T	2.4T	720.0	580.0	460.0	55.0T	30.0
19	14.0T	25.0T	1.1T	960.0	830.0	380.0	240.0	130.0
20	5.6T	3.6T	370.0	320.0	1.9T	230.0	200.0	60.0
21	1.2T	260.0	900.0	1.9T	130.0	280.0	210.0	75.0
22	6.8T	4.8T	6.4M	6.3M	290.0M	270.0M	1100.0M	880.0M
23	250.0	130.0	380.0	3.3T	210.0	280.0	360.0	150.0
24	7.7T	1.1T	100.0T	580.0T	12.0M	4.2M	21.0M	23.0M
25	13.0T	98.0T	1.9M	10.0M	17.0M	53.0M	55.0M	230.0M

[a] Within 1 hr. after inoculation.
[b] Average of ten trials.
[c] Thousands.
[d] Millions.

sources. All trials using milk not aseptically removed from the pasteurizer failed. These failures were attributed to post-pasteurization contamination of the milk with psychrophilic microorganisms, which precluded its use as a growth medium in the pure culture study of similar types. In most of the unsuccessful trials the uninoculated control samples were spoiled by the eighth day of the desired 12-day hold period at 4 ± 1° C.

A comparison of the data in Tables 1 and 2 shows that with these cultures good growth in milk at 4 ± 1° C. was accompanied by an increase in the FFA of that milk. Such increases are attributable only to the lipolytic action of the microorganisms, as no similar increases occurred in the control samples or in samples having little or no growth.

Rancidity and bitterness were the predominant flavor defects (Table 3) developing in inoculated milk samples having good growth with increased FFA, while the flavor of the controls and low-growth samples remained unchanged. Several times during the study rancidity was detected after a very slight increase in FFA and on one occasion as low as 0.9 for one trial with Culture No. 7.

TABLE 2

Increases in the free fat acidity of pasteurized whole milk inoculated with pure cultures of lipolytic microorganisms and held 12 days at 4 ± 1° C.[a]

Culture	Days of storage		
	4	8	12
Controls[b]	0.0	0.0	0.0
1	10.9	45.4	81.3
2	19.0	54.1	76.7
3	6.7	38.6	65.9
4	7.5	39.7	60.2
5	0.0	11.4	32.9
6	1.2	19.8	45.8
7	0.8	3.3	3.3
8	0.1	2.0	6.2
9	0.1	2.2	6.8
10	0.3	3.0	8.1
11	0.2	3.1	9.1
12	0.3	2.2	11.7
13	0.0	0.0	0.0
14	0.0	0.6	2.4
15	0.0	0.7	4.9
16	0.0	0.6	1.6
17	0.0	0.0	0.0
18	0.0	0.0	0.0
19	0.0	0.0	0.0
20	0.0	0.0	0.0
21	0.0	0.0	0.0
22	0.0	1.8	8.5
23	0.0	0.0	0.0
24	0.0	3.6	9.0
25	5.4	46.6	83.4

[a] Each (except for the control) is the average of two trials per culture expressed to the nearest tenth.

[b] Average of ten trials.

TABLE 3

Summary of typical flavor changes occurring in pasteurized whole milk held 12 days at 4 ± 1° C. after inoculation with pure cultures of lipolytic microorganisms

Culture group[a]	Days of storage		
Pseudomonas—I Cultures 1, 2, 3 and 4.	Slightly rancid to rancid	Rancid odor and/or flavor. Sometimes bitter	Rancid odor and/or flavor. Usually very bitter
Pseudomonas—II Cultures 5 and 6	None detectable	Rancid odor and/or flavor. Sometimes bitter	Rancid odor and/or flavor. Usually very bitter
Pseudomonas—III Cultures 7, 8, 9, 10, 11 and 12.	Usually little or no change. Sometimes slightly rancid	Usually slightly rancid. Rarely bitter	Rancid odor and/or flavor. Usually very bitter
Alcaligenes Cultures 13, 14 and 15.	None detectable	Sometimes slightly rancid	Usually rancid
Yeast (unidentified) Culture 16.	None detectable	None detectable	Slightly rancid
Achromobacter Cultures 17, 18, 19 and 20.	None detectable	None detectable	None detectable

[a] Representative of two trials for each of 20 cultures.

Seventeen cultures produced a rancid flavor in milk during the storage period. Six of these developed detectable rancidity by the fourth day, eight others by the eighth day, and the remaining three by the 12th day. With ten cultures, bitterness also developed, but in no instance did it precede rancidity. In only a few trials was bitterness noticeable at the same time that rancidity was first detected.

Brevibacterium lipolyticum (11367), No. 22 was the only organism studied which grew well and brought about measurable increases in the FFA of the milk, yet produced neither rancidity nor bitterness. Its average increase in FFA of 8.5 after 12 days was considerably higher than many of the other cultures where rancidity was detected. Yet in both trials the sample inoculated with this organism was criticized after 12 days of storage as being sour and unclean. This was not expected, since the organism is not characterized as being able to ferment lactose (4). However, in this laboratory it produced a gassy, acid-appearing curd in litmus milk within five days at 21° C.

The data on flavor development presented in Table 3 are a summary compiled from the organoleptic evaluations of the milk samples on the days they were analyzed for microbial content and FFA. It represents the results of two trials for each of the 20 cultures of microorganisms isolated in the laboratory. The cultures are grouped generically, with those in the genus *Pseudomonas* separated into three groups with members of comparable lipolytic ability. This grouping is based on the two-trial, 12th-day average increase in FFA produced by each culture. On this basis, *Ps. fragi* (4973) falls into group *Pseudomonas*—I and *Ps. fluorescens* (11251) into *Pseudomonas*—III.

In general, according to the results of this study, flavor development by pure cultures of lipolytic microorganisms growing well in milk at $4 \pm 1°$ C. follows a fairly definite pattern. The milk first loses its original flavor to become slightly rancid. This is followed by a more distant rancidity, which may or may not be accompanied by bitterness. Usually, if microbial degradation of the milk constituents is allowed to continue, the milk develops an extreme bitterness, which masks rancidity except as detected by the sense of smell. It is doubtful that the bitterness in the latter stage is solely the result of fat hydrolysis as frequently by this time changes in the protein have occurred.

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ULTRA-HIGH-TEMPERATURE SHORT-TIME EXPERIMENTAL STUDIES ON FLUID MILK PRODUCTS. EXPERIMENTAL EQUIPMENT[1]

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SUMMARY

Two heaters have been used to experimentally sterilize small quantities of fluid milk products. The Mallory tubular heater has a capacity of 200 gal/hr and a product flow-rate of 23 ft/sec, but it can be increased to 300 gal/hr. Sections of this heater can be used either as pre-heating, heating or holding tubes for a calculated time of 2.08 sec. each, and they are accurate and efficient, due to the high ratio of length to diameter (2,296:1). The products can be homogenized either before or after sterilization, or both, and over a range of temperatures and pressures.

The DeLaval plate heater has a heating, a cooling, and two regenerating sections which make it possible to homogenize the product at different temperatures. The plates can withstand pressures up to 60 lb/in^2, are 0.043-in. thick with a corrugated profile, and have 1.51 ft.^{2} each of surface. The K factors (B.t.u/hr/ft^2/° F.) for the heating, regenerating, and cooling sections are 700, 300, and 500, respectively. Vacuum- and pressure-steam can be used, depending on the desired temperatures. Two temperature controls are used, one with a vacuum pump in the condensate side of the heating system for about 220° F. and lower, and the other one with a steam trap, to maintain temperatures of about 300° F.

An apparatus was constructed in which milk products can be put aseptically, in sterile glass bottles and in sterile metal cans, either in a normal atmosphere or in one of an inert gas. Detailed dimensions are given.

Ultra-high-temperature, short-time heating of fluid milk products has revealed new research frontiers that need to be investigated for the effects of heat on the palatability, physico-chemical properties, and keeping qualities of these products. In preparing for such studies at the Illinois Agricultural Experiment Station, equipment of small capacity was obtained, altered, and arranged so that experiments could be conducted economically and under controlled conditions. This equipment included a Mallory small-tube heater with an attached homogenizer, a DeLaval plate heater with regenerating and cooling sections, a Manton-Gaulin homogenizer, and an apparatus in which milk can be bottled or canned aseptically, including other specially made utensils. This equipment, and procedures for preparing it for processing sterile fluid milk products, will be described.

Mallory small-tube heater. This heater has been used for experimental purposes (2, 3, 7). More recently (4), it was equipped with a two-stage homogenizer head, which is mounted on an adjustable stand and may be attached at six different positions on the cooling sections, depending on the desired homogenizing temperatures. Since the tubes in the heating and cooling sections will withstand pressures up to about 6,000 p.s.i.g., it is possible to experimentally homogenize milk products at as much as 4,000 to 4,500 p.s.i.g., the difference of

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1,500 to 2,000 p.s.i.g. being the pressure required to pump these products through 143.5 ft. of stainless steel tubing 0.25 in. in diameter in the heating section and the same amount of tubing in the cooling section.[2] It required about 2,000 p.s.i.g. to pump concentrated milk (11.12% fat and 26.73% solids-not-fat) through the heating and cooling sections. A homogenizer, equipped with a 10-h.p. motor, served as the pump. Thus, it was possible, experimentally, to homogenize the same product either before or after sterilization or both before and after sterilization, and over a range of different temperatures and pressures. Furthermore, the product may be heated in Section A (Figure 1) and Sections B and C may

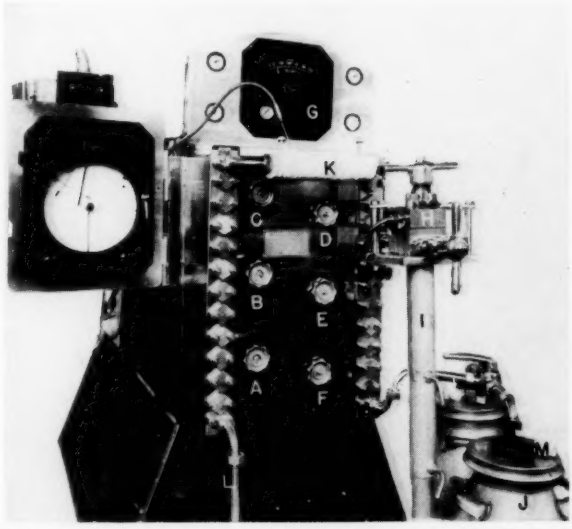

FIG. 1. Mallory heater. A, B, C. Valves for heating sections. D, E, F. Valves for cooling section. G. Steam pressure regulator. H. Two-stage homogenizer. I. Support. J. Sterile can with vent and female connection. K. Flow-line to cooler, containing temperature-recording bulb. L. Feed line from homogenizer-pump. M. Vent on can.

be used for holding it for 4.16 sec.; or Section B may be used for heating and Section C for holding the product for 2.08 sec.; or Section B may be used for heating with only a very short or no calculated holding time, depending on the temperature desired and the product being processed, as the rate of heating depends on the steam pressure and the composition and viscosity of the product. It is possible also to use Section A for preheating milk before sterilization by equipping it with temperature controls, then using Sections B and C for heating and/or holding, respectively. This has been done by manually controlling the flow of

[2] When the homogenizer (H, Figure 1) was installed, 12 ft. of tubes in the cooling section section had to be closed. These closed tubes are about equal to the tubes leading to, to the space within, and to the tubes leading from the homogenizer.

steam into Section A. Sections A, B, and C are equipped with positive individual valves that shut off the steam. Finally, it is possible also to use either one or two or all three Sections D, E, and F for holding the product at a specified temperature by shutting off the coolant, each one holding the product for 2.08 sec. If all of these sections are used as a holding tube, then it would be necessary to install an auxiliary unit to cool the products.

Each tubular section is 47.83 ft. (573.9 in.) long and 0.25 in. wide with eight return bends, giving a length-to-diameter ratio of 2,296 : 1. This ratio is more than five times that stated by Walzholz (9), who described a holding tube in which the ratio of the fastest to the slowest moving particle was 1.25 : 1, the ideal ratio being 1 : 1. Therefore, it can be assumed that each section in the Mallory heater is an efficient holding tube. If the volume were increased to 300 gal. per hour, which is possible, then the holding and heating times would be decreased, proportionately, by 50%.

Since the homogenizer is attached to one of the cooling sections, the product is under pressure through all of the heating sections. For this reason, the fluid milk product can not undergo more than thermal expansion. This retards the adsorption (burn-on) of milk solids on the inside of the tubes. Without this equalization of pressure throughout the heating section, there would be faster adsorption of milk solids, which would show an increased pressure on the gauge of the homogenizer-pump.

The mean calculated velocity of the product at 200 gal/hr was 23 ft/sec through the heating and cooling sections of the Mallory heater, the total time being 12.48 sec. The minimum and maximum velocities at a given instant were not determined. They would be a function of the intervals between thrusts of the homogenizer which served as the pump. At 300 gal/hr, the mean velocity would be 34.5 ft/sec.

The Mallory heater, the attached homogenizer, and three-way valve assembly with male connections, were sterilized by pumping distilled water at 300 to 310° F. through the heating and cooling sections for 10 to 12 min. to sterilize these parts; the outside surfaces of these parts have varying exposures to ambient temperatures.

Gaskets on the headers (return bends) of the heating and cooling sections had to be replaced occasionally. These gaskets were kept in germicidal solutions for 12 to 17 hr. before they were installed. Sometimes during pasteurization or sterilization, small amounts of the milk product would seep past some of the gaskets, due to not tightening the nuts on the headers sufficiently and uniformly or due to excessive amounts of air in the product. When this occurred, the gaskets were replaced because they became a source of post-pasteurization or post-sterilization contamination.

DeLaval plate heater. The plate heat exchanger is shown in Figure 2. It consists of a heating section, two regenerating sections, and a cooling section. Split regenerating sections were used to make homogenization possible at temperatures other than the final heating or final cold regeneration when the product was

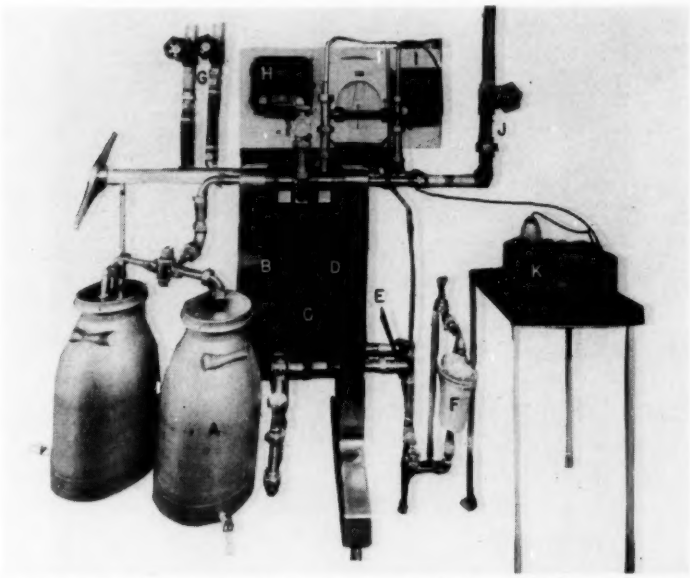

FIG. 2. DeLaval plate heater. A. Sterile can. B. Cooler. C. Regenerator. D. Heater. E. Vacuum steam line. F. High-pressure steam trap. G. Lines for cooling water. H. Steam pressure regulator for 220° F. and lower. I. Steam pressure regulator for 220 to 300° F. J. High-pressure steam line. K. Temperature potentiometer.

homogenized after heating, or final hot regeneration when homogenizing before heating. It is sterilized in the same way as the Mallory heater.

The plates were of the high-pressure type, 0.043 in. in thickness, with a corrugated profile for rigidity and to provide turbulence. The projected plate area was 1.29 sq. ft. and the actual area was 1.51 sq. ft. The distance between the plates was 0.032 in. and the volume of water held between two plates was 0.9 lb. Near their periphery, the plates had a bridging type recess which served as a seat for a synthetic rubber gasket. The maximum working pressure was 60 lb/sq in.

Vacuum-steam and pressure-steam were used as the heating media, depending on the desired temperatures. Two temperature control systems were required: one was operated with a vacuum pump on the condensate side of the heating system and was used at the lower temperatures, and the other required a steam trap to maintain the desired positive pressure on the heating side and was used at temperatures above 220° F.

Typical K factors (B.t.u/hr/ft^2/° F.) were found to be 700 for the heater, 300 for the regenerator, and 500 for the cooler. These values are applicable for milk heated to 200 to 300° F., using 75% regeneration and cooling to 38 to 40° F. The flexibility of the unit is illustrated in the normal rates of flow of 900 to 2,500 lb/hr/stream. It was usually operated at a capacity of 1,720 lb/hr for milk and 1,600 lb/hr for ice cream mix containing 37 to 38% of total solids.

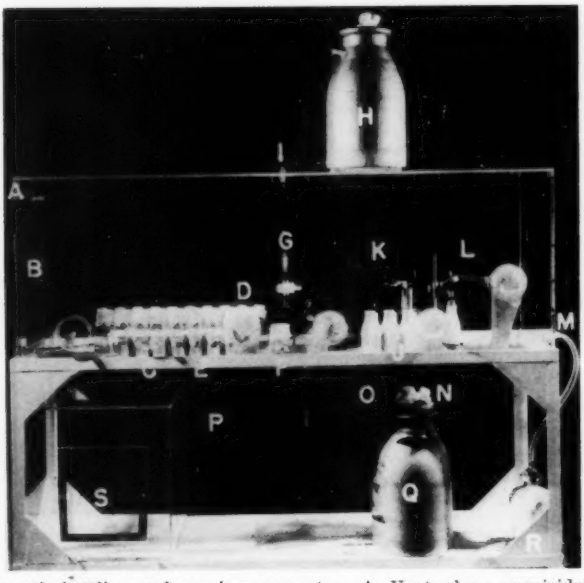

FIG. 3. Aseptic bottling and canning apparatus. A. Vent where germicidal solutions are sprayed into chamber. B. Opening through which equipment is placed in chamber. C. Attachments for rubber gloves. D. Glass bottles. E. Metal cans. F. Caps for bottles. G. Milk dispenser. H. Can for milk product. I. Connection to can. J. Filled bottles. K. Can sealer. L. Bottle capper. M. Air and/or gas line. N. Vent tube on can. O. Female fitting on can. P. Chute. Q. Sterile can. R. Filter. S. Compartment for holding filled cans.

Containers for collecting milk from the heaters. Ten-gallon cans, made of stainless steel, were used to collect milk from the cooling section of the heaters (Figures 1, 2). A gum-rubber tube about 3 in. long was attached to the outlet of each can. (I, Figure 3.) The end of this tubing was wrapped with parchment, which overlapped about 1.5 in., then it was fastened with a rubber band at the base and left open during sterilization. A layer of absorbent cotton, 2 in. wide and about 0.5 in. thick, was inserted between the outer and inner rim of the cover which was placed loosely on the can. The intake tube (O, Figure 3) with a sanitary connection was covered with cotton, then with parchment, and fastened with a rubber band. The vent (N, Figure 3) was also covered with cotton, which was fastened with a rubber band. The 10-gal. cans, with their covers loosely attached, were sterilized in the autoclave at 248° F. at 15 or 16 p.s.i.g. for 50 min. Immediately after, they were sterilized, the covers were securely fastened with wire to the handles of the can, and the parchment on the outlet tube was folded back 180° and fastened with a rubber band. When the cans had cooled, the rubber tube was closed with a pinch-cock.

Aseptic bottling and canning apparatus. Attempts were made to fill bottles with fluid milk products directly from the cooling section of the Mallory heater. Unfortunately, the bottles could not be filled uniformly and microorganisms in the

air infected the milk products. Therefore, it was necessary to construct an apparatus in which the milk products could be put in glass bottles or metal cans, aseptically. This apparatus was made of lucite, the joints of which were held together with screws 1.5 in. long and, in addition, these joints were sealed with lucite glue. This glue was made by dissolving 10 to 12 g. of finely ground lucite in 100 ml. of methylene dichloride, which flowed between the joints before the screws were tightened. This aseptic apparatus had a trap door, connected to a chute, through which cans of milk rolled to a lower compartment as soon as they were filled. The construction of parts, dimensions, and location of the different openings are shown in Figure 4. This was an improvement over the apparatus described previously (3).

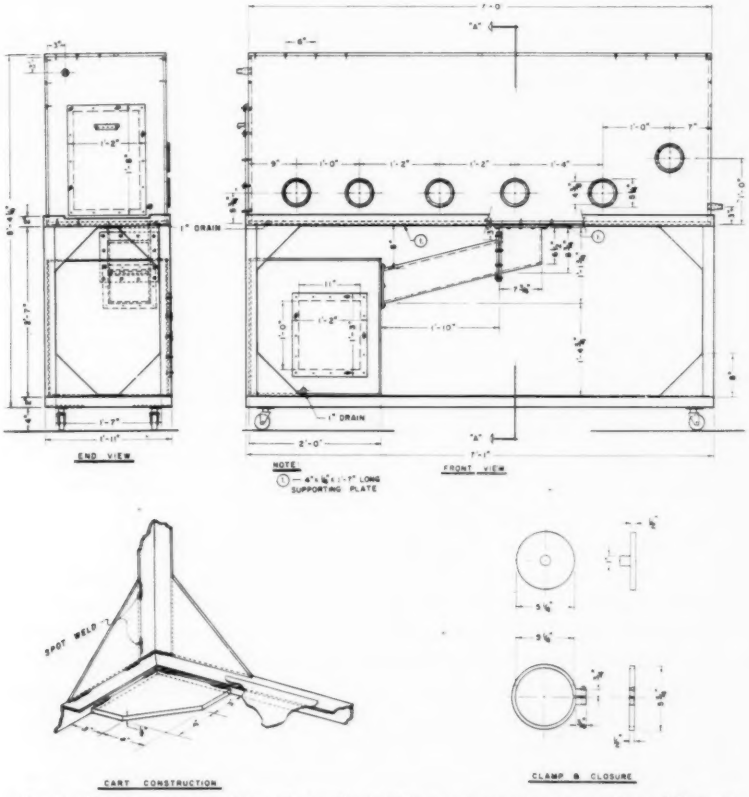

FIG. 4. Showing construction of aseptic bottling and canning apparatus with cart.

The bottling apparatus was supported on a cart, the frame of which was made from 2-in., 90° angle iron, 0.25 in. thick and reinforced at the corners with welded iron plate of the same thickness. This cart was mounted on four swivel-

type, rubber-covered wheels, 3 in. in diameter, which could be locked in place. A platform was provided for utensils and accessories. The entire apparatus could be moved into an air-cooled room.

Twenty gallons of a fluid milk product could be bottled or canned in one operation in containers of various sizes. These containers could be filled in an inert atmosphere by displacing the air with carbon dioxide or nitrogen gas and maintaining a slight positive pressure with these gases. It was necessary to sterilize the bottling and canning apparatus, the bottle capper, the can sealer, and the rubber gloves with germicidal solutions. The rubber gloves were dipped in solutions of either 500 p.p.m. of chlorine, quaternary ammonium, or hydrogen peroxide, and were attached with clamps to openings provided for them (C, Figure 3). Next the apparatus was sprayed through Opening A for about 1 min. with one of the germicidal solutions with an air-pressurized sprayer. The sterilized bottles and caps were placed inside the apparatus. An aluminum rod, the end of which was bent in a half circle, was also placed in the chamber and it was used to move empty and filled bottles or cans. The milk dispensing assembly was put in the operating position and the apparatus was again sprayed with the germicidal solution for about 30 sec. The filtering assembly was connected to a compressed air line and air was passed through the apparatus to remove the excess germicide. The door (B, Figure 3) was left partially open during this aeration. Schulz (6) has described a similar apparatus for bottling milk aseptically.

Milk dispensing assembly. The milk product in the 10-gal. can flowed through a glass tube, 15 mm. inside diameter, and into the asepticized apparatus, where it was put in glass bottles or in metal cans. This tube was fitted with 19/38 tapered, ground glass joints at its upper end and the female part had a tube 3 in. long, bent at 90°, connected to the 10-gal. can on top of the apparatus (I, Figure 3). A glass stop-cock was connected with 19/38 tapered joints to the lower end of the tube (G, Figure 3). Before sterilization, and to protect from contamination afterwards, parchment was rolled around and about 1.5 in. beyond the upper end of the tube and also at the end of the stop-cock. After sterilization, the parchment at both ends was folded back 180° and fastened with a rubber band.

Because of greater durability, the glass dispensing assembly was replaced with a stainless steel tube of the same inside diameter as the glass. It was bent 90° at the top to connect with the 10-gal. can and a glass tube was attached with a rubber tube at the lower end. The flow of fluid milk products into containers was regulated with a pinch-cock.

Milk containers. Glass bottles of pint and quart capacity were used. They had a specially made rim, 38 mm. inside and 42 mm. outside diameter. In some experiments, beverage bottles with opening 18 mm. inside and 26 mm. outside diameter and of clear, brown, and ruby glass were used. They were closed with friction crown caps. Metal cans of various sizes also were used.

The closures of the glass bottles and of the metal cans were covered with parchment, held in place with rubber bands. The metal caps for the bottles

and cans were placed in beakers covered loosely with parchment. They were sterilized for 40 min. at 15 to 16 p.s.i.g., then the parchments on the beakers were fastened securely with rubber bands as they were removed from the autoclave.

Bottle capper and can sealer. The stainless steel bottle capper, manually operated, was made in the University machine shop. It closed and crimped tightly the metal crown caps on beverage bottles of different sizes. It was used also to press the friction-type metal caps on to the closures of the milk bottles. The capper was fastened with bolts to the floor of the aseptic apparatus (L, Figure 3).

A can sealer operated by an electric motor was used to seal covers onto the metal cans.[3] It was not fastened permanently in the aseptic apparatus (K, Figure 3). There was room for both the bottle capper and the can sealer in the aseptic apparatus.

Air and gas filter. The air and gas filter for the aseptic bottling unit was made from a four-liter dispensing jar, filled with cotton and equipped with a clamp to hold the rubber stopper. This jar was connected to the air or to inert gas supplies and to the aseptic apparatus with plastic tubing, 20 mm. inside diameter. The two openings in the jar were plugged with cotton and the filtering assembly was sterilized in the autoclave for 50 min. A pressure of 30 p.s.i.g. was used to force air or inert gases through the filter and into the aseptic apparatus. The plastic tubing was sterilized with hydrogen peroxide. Several variations of the filter were used before this one was adopted (R, Figure 3).

The term aseptic is used to indicate that air-borne microorganisms were eliminated as sources of contamination. The vegetative organisms were destroyed by the chlorine, quaternary ammonium, or hydrogen peroxide germicides, but the spores probably remained dormant on the inner surfaces of the apparatus. However, hydrogen peroxide will destroy certain species of spores (5), though studies have been limited to a few species in milk with varying concentrations of the germicide.

To determine if it were possible to prevent air contamination in this apparatus, about 20 qt. of nutrient broth were poured into the 10-gal. can and sterilized in the autoclave, cooled, and bottled in the asepticized apparatus. Some bottles were filled completely, others to varying degrees of fullness. They were incubated at 70 ± 0.5° F. for 76 hr., then at 100 ± 2° F. for 97 hr. The broth remained clear, indicating no visible growth. Twelve bottles were selected at random and samples of broth from each one were plated by Standard Methods (1). No growth was observed on the Petri plates. In the same way, about 20 qt. of milk were sterilized, bottled, stored, sampled at random, and plated. No bacterial growth was observed. Removing the closures from the bottles of broth and milk, and exposing them to the air, resulted in the growth of air-borne microorganisms. Repeated trials have shown that sterile milk can be put aseptically

[3] Automatic Canning Devices, Inc., Manitowoc, Wisconsin, made this apparatus.

into glass bottles and into metal cans. Research results obtained with this equipment have been published (4) and other results will be forthcoming.

ACKNOWLEDGMENTS

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SUMMER FEEDING OF DAIRY CATTLE. A REVIEW[1,2]

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SUMMARY

Income for dairy farmers during the summer depends to a considerable extent on more pasture herbage production in hot-dry weather. The results of recent studies indicate that dairy cattle fed an adequate ration, and allowed to graze nights and days with shade during the days, are far less affected by high environmental temperatures than previously supposed. Increased quality and quantity of forage may be enhanced by: (a) growing crops more resistant to heat and drought, (b) maintaining more moisture in the soil by less defoliation of plants which give shade to the soil, (c) irrigation, and (d) fertilization of the soil (especially with nitrogen, phosphorus, and potassium). The problems involved in using animal production (growth, milk yields) in pasture evaluation were pointed out. Indirect methods of pasture evaluation using internal and external indicators were described. A combination of internal and external indicators makes possible the estimation not only of the chemical composition but also of the coefficient of digestibility and dry matter intake of pasture. Dry matter intake from pasture is variable, but usually is about 30 to 35 lb. per day for cows weighing 1,200 lb. Grazed pasture herbage is higher in crude protein and lower in crude fiber and higher in digestibility than comparable hand-fed herbage. The requirements of grazing cattle are about the same as in barn-feeding, with the exception of digestible energy. Grazing cattle require from 1 to 3 lb. of TDN per cow per day more than those barn-feeding. Losses of pasture herbage from trampling, when properly managed, are about 10 to 15%. These losses are much higher with tall pastures. Temporary pastures, such as Sudan grass, are needed in many areas to supplement perennial pastures during the summer. The success of different systems of pasture utilization hinges on proper management (proper stocking rate, use of high-producing cows). Rotational and strip-grazing systems aid in using intensive pastures. Soilage may be used to advantage on farms where good pasture land is not conveniently located. Where soilage and stored feed may be used to advantage was pointed out. The fermentation vat of calves fed feeds other than milk alone is sufficiently developed at 2 to 4 mo. of age to handle good pasture, but the calves will starve on poor pasture. Certain pasture forages, such as Sudan grass, may contain considerable hydrocyanic acid. When properly managed, however, the danger from prussic acid poisoning is not great. Although the cause of pasture bloat is not known, recent results indicate that the administration of penicillin prevents bloat for a short time. Also, vegetable oils sprayed on "bloaty" pastures, added to the drinking water or to the feed, reduce the incidence of bloat. Milk produced by cows on pasture is more resistant to oxidized flavor than when winter feeds are fed. Pasture feed flavors may be avoided by removing cows from pasture 3 to 5 hr. prior to milking.

One of the most important problems facing the dairy farmer in many areas of the world is the decline of milk production during the summer months. The critical period between the abundant pasture supply in spring and the revival in the fall is associated with high temperatures, low humidity, and insufficient rainfall. Profitable dairy production is based on the ability to utilize known information about management of both cattle and forage crops. This is especially important in overcoming the decrease in pasture production, which results in a decline in milk yields, and the increase in feed costs due to the feeding of harvested herbage and concentrates.

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This review is primarily concerned with the economics of pasture feeding, the effects of summer climate on milk production, pasture crop production, changes in nutritive value of forage crops, methods of measuring pasture production, the uses of pasture, and the relation of pasture to disease and milk quality. No attempt will be made in this review to cover all of the areas of summer pasture production, such as the breeding of forage plants resistant to drought and high temperatures, or the value of different species of permanent and temporary pasture crops. The role of the fermentation vat in herbage utilization, as well as plant metabolism dealing with reduced digestibility with maturity, will not be discussed in the review. References to many reviews are included in lieu of a more complete literature coverage.

ECONOMIC IMPORTANCE OF PASTURE

The economics of pasture feeding compared with feeding harvested crops appears to justify the use of land for pasture similar to that now used for cultivated crops (303). Unfortunately, the data for the economics of pasture usually include the entire pasture season, although it is well recognized that pasture herbage is worth more during the summer months, in most areas, than that produced in the spring or fall.

The data relative to the cost of producing 100 lb. of TDN from various crops grown in 16 states are shown in Table 1 (312). Ahlgren *et al.* (4) concluded that pastures offer the livestock producer his cheapest and most economical source of feed. When cows were pastured from mid-May to mid-October, there was a reduction of 46% in the amount of grain fed, 86% in roughage fed other than pasture, and 31% less labor used daily in caring for the cow (75). The average reduction in feed and labor costs per cow per day, after charging the cost for pasture, was 48% below that for the winter-feeding period.

Studies in New Jersey (survey) showed that pasture was the cheapest source of TDN (49). The costs per 100 lb. of TDN from different sources were: pasture, $1.26; hay, $1.74; silage, $2.07, and concentrate feed, $4.23. The studies showed that many farmers were getting only about 500 lb. of TDN from each acre of pasture. Better-managed pastures, however, yielded 1,500 lb. of TDN per acre.

TABLE 1

Cost of producing 100 lb. of total digestible nutrients from various crops (312)

Crop	TDN/acre	Cost/100 lb.
	(lb.)	*($)*
Oats	932	2.02
Wheat	1,146	1.88
Barley	1,217	1.70
Corn silage	2,320	1.54
Corn grain	1,778	1.38
Timothy hay	1,257	1.21
Clover and timothy hay	1,347	1.15
Soybeans	1,725	1.06
Red clover hay	1,622	0.97
Alfalfa hay	2,522	0.83
Pasture	.....	0.64

The unit cost of producing milk on farms near Chicago was considerably less in summer than in winter (305). The cost of producing 100 lb. of milk in summer (May-September) was $1.41, vs. $1.82 in winter (October-April). The cost of producing milk was lowest during June and July and highest in January, February, and March. In January, the feed cost for producing 100 lb. of milk was nearly twice as much as in June, when the cows were on pasture. Similar results were reported for the St. Louis milk shed (306).

According to an Indiana study, pastures did not produce as much feed per acre as when harvested crops were grown on the land (315). When the expenses for labor and machinery for planting, tilling, and harvesting crops are considered, the net return from pasture may exceed the net return from harvested crops on the same land. Wright and Hodge (313), in a study of milk production costs in southeastern Michigan, concluded that pasture at $21 per cow for the season provided nutrients at about one-half the cost of harvested roughage. A pound of TDN provided by concentrates cost 3.6¢, whereas the cost per pound of TDN provided by pasture was only 1.1¢.

Morrison and Ely (187) found over a 10-yr. period that the average TDN yield of bluegrass pasture was 2,226 lb. per acre. The labor involved in the production of pasture was far less than that required for the production of corn or alfalfa hay, while the TDN yield of the pasture was fairly comparable with that of corn and alfalfa hay grown in the vicinity of Lexington, Kentucky. In a comparison of low- and high-yielding cows, Vogel and Schott (292) showed that there was a loss during winter-feeding period; whereas, during the pasture period there was a profit for both high and low milk producing cows.

It is apparent that the use of well-managed pastures offers the dairyman an opportunity to increase his income.

EFFECTS OF CLIMATE ON CATTLE AND PASTURE CROPS

The summer feeding of dairy cattle involves a wide range of climatic conditions from almost ideal temperature, humidity, and rainfall to very hot and dry conditions. It is important for the dairyman to know the effects of adverse climate so that he can use improved methods for managing cattle and herbage crops under these adverse conditions.

Effect on cattle. Data have recently been reviewed concerning the effect of high environmental temperatures and humidities on the composition and yield of milk (92, 135, 246). Most of the results showed a decline in the milk yield and a change in the composition of milk when the environmental temperatures were over 80° F. but, in some cases, the adverse effects of high environmental temperatures were not pronounced (246). The effect on the composition of milk when cows are changed from barn to pasture was reviewed by McClymont (168). A depression in the fat content of milk resulted from the consumption of short, lush pastures plus fairly high levels of concentrates. The grazing of leafy oat pasture supplemented with concentrates, with limited amounts of other roughage, resulted in a depression of the butterfat percentage. These results, however, were probably not due to high environmental temperature but to high concentrate feeding.

The importance of a good ration during hot summers is indicated by Rhoad (232), who suggested that the loss of energy is, in a large way, responsible for the low production records of European cattle in the tropics. Rupel and Leighton (245) showed that cows fed a high fiber ration during hot weather produced less milk than when fed a low fiber ration. Since the two rations were balanced on a net energy basis, and not on a TDN basis, the extra heat increment produced by the high fiber ration may have been difficult to handle in hot weather. Peters *et al.* (207) reported that cows were apparently cooler on a low fiber ration because of lower body temperatures, slower respiration rates, and slower pulse rates. Holstein cows also produced milk higher in fat and solids-not-fat when fed a low-fiber ration compared to a high-fiber ration.

Using climate-controlled rooms, the Missouri workers (35, 36, 218) have found that the zone of thermal neutrality for milking cows lies between 30 and 60° F., depending on the breed, production level, and other metabolic factors. At high temperatures, humidity also becomes a very important factor, since more of the heat loss of the animal occurs through the skin and lungs. Ragsdale *et al.* (220) reported a drop in milk production with increasing environmental temperature beginning at 80° F. for Holsteins and at 85° F. for Jerseys. The decline in feed consumption with increased environmental temperature about paralleled the decline in milk production. The decline was steeper for the Holsteins than for the Jerseys.

In Puerto Rico, Basherov (25) found that the Guernsey breed is well adapted to subtropical conditions in respect to milk production, reproduction, breeding efficiency, and growth rates. Arrillaga *et al.* (15) reported that the Jersey, Brown Swiss, Guernsey, and Ayrshire breeds, in this order, are adapted to Puerto Rican climate and environment as shown by their body reactions and milk production. The Holstein breed seems to be less adaptable than the other breeds. These investigators concluded that there is nothing in the Puerto Rican environment to prevent the future development of the dairy breeds, if suitable methods of feeding, management, and protection against disease and parasites are employed. Similar results were reported by Riek and Lee (237), who exposed Jersey cows 7 hr. twice a week to temperatures ranging from 85 to 110° F.

According to Johnston (135), temperate climates usually have a 20° F. or greater difference between daily maximum and minimum temperatures. The lower night temperatures permit night grazing. Seath and Miller (255) found that cows graze about 75% of the time at night during hot weather. Payne *et al.* (205) found that cows graze 67% of the total grazing time at night. The total grazing time diminishes slightly during the hot months in the tropics. The lower night temperatures allow the cattle to recover from heat stress experienced during the day. In climate-controlled chambers, there is a tendency for milk production to decline with high environmental temperatures. When cows are allowed to adapt to hot weather gradually, as in natural climate in many hot-weather places, the reduction in milk production is delayed for about a month and then occurs only gradually (135). Johnston stated that the principal cause of "summer slump" in milk production is probably due to a decline in quantity

and quality of available herbage, probably aggravated by poor management practices. High body temperatures alone do not necessarily result in lower milk production if the animals are fed adequate TDN and their appetites remain normal. The importance of excellent forage to maintain milk production in hot weather was pointed out.

The milk production of cows kept indoors at night was less than that of cows grazed at night and day. The average herbage intakes for cows grazed day-and-night and day-only were 187 and 150 lb., respectively (291).

Cows kept in an air-conditioned chamber at 70° F. produced more milk than cows kept in an open shed (shade) when the maximum temperature was 92.7° F. (136). Roughage consumption was not affected in this study.

Shade and sprinkling. The effects of shade and sprinkling water on dairy cattle during hot weather were reviewed by Seath and Miller (254). Exposure of cows to sunshine (83–90° F. and relative humidity 61 to 80%) for 2 hr., then put in the shade, resulted in a reduction in body temperature. Sprinkling cows (body temperature 102.5° F.) reduced the body temperature by 0.54° F. after 0.5 hr. in the shade, and 1.1° F. after 1 hr. They concluded that either shade alone, or sprinkling followed by shade, was effective in reducing body temperature, respiration rate, and pulse rate. Seath and Miller (256) also showed that when cows were removed from sunshine, sprinkled with water, and then subjected to a gentle breeze, body temperatures were reduced rapidly. This may explain why rain and wind tend to cool milking cows during the summer.

Water supply. One of the problems in feeding dairy cattle in summer is the assurance of an ample supply of drinking water. This subject has been reviewed by Leitch and Thompson (159) and by Sykes (275). Cattle get considerable water when large amounts of succulent pasture forage are consumed. The total water intake, therefore, includes the water in feeds. It has been shown by several investigators that, in general, water intake follows dry matter consumption, although attempts to correlate the two have not been satisfactory.

Recent investigations at the University of Missouri include water consumption data, together with information on feed intake, environmental temperature, and relative humidity (210, 220). The effect of relative humidity on water consumption of dairy cattle was negligible at temperatures above 75° F. Winds up to 9 miles per hour did not affect water intake (35). The amount of free water consumed by Jersey cows on New Zealand pasture during the summer averaged 5.9 gal. per day, with a range of 1.4 to 11.5 gal. (50). Water intake data of milking cows at various temperatures, body weight, and butterfat percentages are shown in Table 2 (307).

Effect on pasture. The literature pertaining to the effect of fluctuations in weather upon the grasslands was reviewed by Aamodt (1), Coupland (64), Black (31), and Mitchell (182), who found a close relationship between the reduction in forage yield and drought conditions, that yield was closely related to soil moisture, and that forage yield was closely related to the ratio of precipitation to evaporation. The significance of leaf area in pasture growth was pointed out by Donald and Black (73).

TABLE 2
Water requirements of cows producing 50 lb. of FCM daily, in gallons (307)

Body weight	Temperatures (° F.)					
	40	50	60	70	80	90
(lb.)						
800	17.2	18.0	20.3	22.8	25.3	31.6
1,000	17.8	18.8	21.1	23.8	25.4	33.2
1,200	18.5	19.5	22.0	24.7	27.5	37.1
1,400	19.5	20.6	23.2	26.2	29.2	37.1
1,600	20.2	21.3	24.0	27.2	30.3	38.7

Owens (202) reviewed the papers presented at the Seventh International Grassland Congress held in New Zealand, which pertained to the effects of moisture, temperature, and light on the growth of herbage plants. It appears that plants continue to take up moisture from a soil that is getting drier, but that their ability to do so is impaired by close grazing. Such plants lose two ways; first, from loss of leaf surface for photosynthesis and, secondly, from lack of moisture. The most effective way to combat over-dry conditions on pasture herbage is to strip-graze so as to leave some pasture intact.

Investigations have shown that the mineral and protein contents of pasture plants vary widely according to species, temperature, amount of light, stage of growth, soil type, and fertilization (234, 260, 261, 272). Richardson *et al.* (234) pointed out that the protein and mineral contents of pastures from tropical and subtropical areas, in general, are lower than for pastures in temperate regions. He also stated that climatic factors affect the protein and mineral contents of a pasture; and the differences in mineral content of pastures in tropical and temperate regions may be caused by a differential effect of climate on the rate of nitrogen and mineral uptake on the one hand, and the rate of growth on the other. In tropical and subtropical climates, the growth of pasture is rapid, and frequently the plant approaches maturity within a few months after the onset of the wet season. In cool, temperate climates, growth proceeds much more slowly and the productive stage is reached only after months of slow vegetative growth. According to Harris (100), improved pastures mitigate the effects of drought and are responsible for the commencement of a soil fertility cycle by which the organic content of the soil is built up, the physical condition is improved, moisture retention is improved, and a layer of humus collects on the surface to reduce evaporation. This upward trend in the soil fertility cycle permits a wider range in the selection of plant species and results in a more rapid regeneration of pastures.

Todd (279) investigated the effects of rainy and dry seasons on the chemical composition of herbage in Kenya. Protein and phosphorus dropped to very low levels during dry periods. The studies of Reid *et al.* (228) indicate that the TDN value of herbage and, to a lesser extent, the amount consumed declines during prolonged dry periods, but increases sharply following heavy rains. Climatic conditions appear to be important determinants of quality of forage.

Irrigation. The literature dealing with the use of supplemental irrigation for the production of pasture herbage was reviewed by Trumble and Walker (284). Peterson and Hagen (208) stated that timely application of water in the correct amount is necessary to maintain summer pasture production in many parts of the world. Bateman and Keller (27, 28) reviewed the literature pertaining to irrigated pastures in arid regions and pointed out that a dairyman with a productive herd, coupled with proper management, could make a good pasture highly profitable by the use of irrigation. The establishment of more productive irrigated pastures has been a rather slow process, because it has involved the use of pasture on the better farm lands and the application of a completely different concept of pasture management—rotation grazing or strip grazing. They also pointed out the importance of using a proper pasture mixture, not only from the standpoint of nutrients but also from the standpoint of palatability. Grazing animals generally prefer younger plants to older plants, but the rate of decline in palatability with maturity varies greatly for the different species. Forage must be sufficiently palatable to be acceptable to the grazing animal, and the various components of a mixture must be utilized to such an extent that they can be maintained in proper balance throughout the life of the pasture. Species which are relatively low in palatability tend to increase with time in response to differential grazing.

Bateman and Keller (27) found that frequent applications of irrigated water are better than heavier, less frequent applications for pastures. Ladino clover, being shallow-rooted, will not persist in pasture where irrigations are not fairly frequent. During their study, pastures wer irrigated five to seven times a season. In one 5-yr. period, the average number of days between applications was 26, 18, 13, 19, 17, and 18, from the first to the last, with an average of 3 to 4 in. of water per irrigation. This requires putting water over the field as fast as possible, but never when cattle are on it. The importance of maintaining the productivity of irrigated pastures through a number of seasons was stressed. In this study, pastures were grazed by cows that averaged approximately 10,900 lb. of FCM per cow per year. In another experiment, milk production for a 9-yr. period averaged 7,277 lb. of FCM per cow. Another pasture produced 8,044 lb. of FCM per acre, for a 5-yr. period. These pastures received adequate and properly spaced irrigations, applications of manure and phosphate fertilizer, and the forage was allowed to make a good recovery between grazings.

Experiments conducted by Van Horn *et al.* (289) in Tennessee gave results applicable to a large area of the United States where dry periods are frequently encountered during the summer months. The irrigated pasture produced 61% more grazing, as indicated by calculated yields of TDN, and 58% more milk than the unirrigated pasture. Distribution of grazing was greatly improved by irrigation. At the end of 4 yr., a good stand of Ladino clover remained in the irrigated pasture, but the orchardgrass was about one-fourth as thick as in the unirrigated pasture. Summer droughts are common in the region where this experiment was conducted, but the droughts which occurred while the experiment was in progress were longer and more frequent than normally would be

expected over a long period of years. Differences between irrigation and no irrigation during a series of more favorable years would probably be less than those obtained in this experiment. The authors also pointed out that farmers have other methods for maintaining or increasing milk production. Instead of investing in irrigation equipment, money might be used to provide silos for the production of additional silage for use during the periods when pastures are inadequate, or for the purchase of additional feed. The profitableness of alternative methods of increasing milk production would depend on individual farm conditions. Many farmers do not have a source of water available for irrigation.

Staten *et al.* (268) emphasized that irrigated pastures under New Mexico conditions must produce at a maximum and that rotational grazing is essential. The practice of spreading the droppings is one of the most important items in pasture management. Failure to spread droppings may result in loss of as much as one-third or more of the grazed area. Weeds and unconsumed forage should be mowed.

The results of a 10-yr. experiment on irrigated pastures at Huntley, Montana, showed that the carrying capacity of the pasture varied from 1.98 cows per acre in 1919 to 2.73 cows in 1927, with an average of 2.2 cows (148), and that an average of 6,990 lb. of milk and 266.7 lb. of butterfat were produced per acre of pasture (138.4 days of grazing). No grain was fed during the pasture period.

Tesar *et al.* (276) and Hoglund *et al.* (114) of Michigan found that the application of 3⅓ in. of water annually to an alfalfa-Ladino clover-brome-grass pasture resulted in 17.9% increase in milk production over the control pasture. The increases in milk production resulted entirely from greater carrying capacity of the irrigated pasture and were not the result of increased production per animal. Grain was fed at the rate of 1 lb. for each 4.4 lb. of milk. They concluded that in sections of the humid eastern United States, where the replacement value of hay is likely to exceed $25 per ton, irrigation might be profitable, if increases in forage production were similar to or greater than those obtained in these experiments. Irrigation of a dairy pasture may also be valuable as a means of increasing milk production per acre near metropolitan areas where land is scarce.

Munro (188) reported that the utilization of the herbage by young stock (15 to 18 mo. of age) was significantly better with irrigation. Irrigation improved the botanical composition of the pasture; the clover percentage increased on the irrigated plots, but the application of nitrogen produced some depression. This depression increased with increased moisture deficit. Milton (181) found that some species responded more than others to irrigation, and stated that timothy, ryegrass, and white clover produced relatively more growth and denser herbage under irrigation than when not irrigated. The reverse was true of cock's-foot. Munro (188) showed that nitrogen application, except for spring and fall, may be wasted on nonirrigated grass in a dry summer. The over-all increased utilization of the irrigated plots was highly significant. Munro attributed this to an increase in the palatability of the herbage and to the more

rapid breakdown of organic matter which would prevent fouling of the herbage by old dung "pats". These dung pats broke down and disappeared quite rapidly on the irrigated plots. The dung pats on the unirrigated pasture remained hard and dry throughout the summer and little breakdown was observed. Earthworms were much more active on the irrigated plots and were believed to aid in the quick breakdown of dung pats.

As pointed out by Bateman and Keller (27), one of the keys to increasing the milk production of irrigated pastures is the application of fertilizers.

Pasture fertilization. Boyd and Lessells (32) and Ward (299) reviewed the literature dealing with the fertilization of grassland. Brown and Munsell (38) showed that the protein content of timothy declined with maturity. Nitrogen fertilization increased both the percentage of protein and the yield of hay. Alfalfa out-yielded timothy, regardless of the nitrogen treatment used. In pasture-clipping studies of Ladino clover, Kentucky bluegrass, and Rhode Island bent grass, grass-legume mixtures out-yielded the grasses that had been fertilized with nitrogen in both total yield and protein. Carey *et al.* (51) reported that the fertilization of bromegrass with nitrogen resulted in a higher nitrogen percentage in the forage.

The application of nitrogen increased the yield of grasses and usually the protein content was increased. For instance, Watson *et al.* (302) stated that grassland pastures in England yielded 60% more forage from nitrogen fertilization than from no fertilizer treatment. The percentage of protein was increased by nitrogen fertilization. Also, nitrogen fertilization resulted in 13 days' earlier spring grazing and 15 days' later fall grazing than are normally obtained. Holmes (117, 118) reported that yields of grasses in England showed a year-to-year decline following continued high applications of nitrogen at rates of up to 520 lb. per acre. This decline was attributed to a potassium deficiency, brought about by the continued removal of large amounts of forage as a result of earlier application of nitrogen. Watkins (301) found that in order to obtain maximum yields from the application of urine, it must be applied to grass and not legumes. Application of phosphorus and potassium in adequate amounts is also essential for maximum herbage yield.

According to Barker (21), the productivity of grassland is largely determined by the availability of the soil nutrients within a particular environment. These can be regulated by fertilizer application, but nitrogen has the most potent influence on grass production. With good management of both land and cows, the amount of feed per acre can be increased by nitrogen fertilization at an almost linear rate up to more than 100 lb. of nitrogen per acre. Holmes and Aldrich (120) emphasized that there is plenty of evidence to indicate that cheap cattle feed can be produced by the efficient use of nitrogen on grassland. Full exploitation of this knowledge depends on a mastery of the techniques of grass utilization. Davies (68) pointed out that in New Zealand and the United States the tendency is to depend on legumes to supply a part of the nitrogen to forage crops, while in Holland and Great Britain the tendency is to rely on nitrogen fertilization. Both systems provide high yields of herbage. This problem was investigated by Wagner (293).

Levy (158) of New Zealand showed that it was possible by proper management to produce under all-year grazing 14,000 lb. of pasture dry matter containing 20 to 30% protein, and with 7,500 lb. of starch equivalent per acre per year. This is sufficient nutrients for the production of 500 lb. of butterfat per acre. A pasture producing 14,000 to 15,000 lb. of dry matter annually requires about 500 lb. of nitrogen, 1,700 lb. of potash, 7,500 lb. of superphosphate, and 300 lb. of lime per acre. He cited the work of Sears (248), which showed that a high-yielding New Zealand pedigree clover will make available from the air 400 lb. of nitrogen per acre annually, but depleted materials must be supplied.

Holmes (118) summarized the results with heavy application of nitrogen upon pasture yields at the Hannah Dairy Institute in Scotland under good management conditions. The addition of nitrogen in 100-lb. increments up to 350 lb. per acre showed a straight-line increase in dry matter yield, and only a slight decrease in efficiency of dry matter production up to 500 lb. per acre. Holmes concluded that increased yields per acre of dry matter, Starch Equivalent and protein are almost linearly related to nitrogen supply up to about 300 lb. per acre. Clover pastures can contribute the equivalent of 150 to 200 lb. of nitrogen per acre. Each cow contributed 0.5 lb. of nitrogen daily in the form of urine and feces, or nearly half of the required nitrogen. Holmes stressed that if these results are to be obtained under practical conditions the efficiency of utilization must be high. Rotational pastures are essential, strip grazing is desirable, and good conservation methods are required. Holmes and Aldrich (120) compared the cost of Starch Equivalent produced from the use of nitrogen fertilizer on grass with the cost of other feeds and concluded that cheap cattle feed can be produced by the efficient use of nitrogen on grass.

Ramage *et al.* (221) of New Jersey applied nitrogen in 50-lb. increments up to 450 lb. per acre. The dry matter yield at 50 lb. per acre was two tons containing 12% protein. These yields gradually increased with each 50-lb. increment up to 4.5 tons, containing 20% protein at the rate of 450 lb. of nitrogen per acre. Nitrogen application of 100 lb. per acre gave the greatest yield of dry matter and protein per pound of applied nitrogen.

Sprague and Garber (267) studied the effects of various harvest treatments and nitrogen fertilization on Ladino clover grown in combination with orchardgrass and smooth bromegrass. The application of 60 lb. of nitrogen per acre increased grass yields and reduced clover growth. The fertilization of pastures with nitrogen, phosphorus, and potassium increased forage yield (147). Crude protein, phosphorus, and potassium percentages in the forages were increased. Mid-season application of nitrogen increased the nutritive value of the forage. Soil types had greater effect on the chemical composition of the forage than did the application of lime, nitrogen, and phosphorus as fertilizers (286). Phosphorus and potassium fertilizers tended to increase clover population. Forage yields from grasses were greatly increased by nitrogen fertilization (274). The importance of using fertilizers on pastures was pointed out by Abell (3).

According to Holt *et al.* (123), the application of fertilizers to both summer annual and perennial grasses resulted in increased yields. Nitrogen, phosphorus,

and potassium were found to be necessary for good pastures in Texas. The effects of nitrogen fertilization on composition and dry matter production of grassland were also investigated by Ferguson and Terry (82).

In a long-continued hay-pasture experiment in Connecticut, lime, phosphorus, and potassium were necessary for the maintenance of high proportions of volunteer white and red clovers (37). The quality of forage from the nitrogen-treated plots was inferior to that from the more clover, no-nitrogen area, according to the preference of yearling heifers. Attention was called to the importance of legumes in grassland farming which has been emphasized by many investigators. There is no doubt that legumes save tremendous amounts of fertilizer nitrogen. The dry matter and protein yields of Kentucky bluegrass and Rhode Island bentgrass, lawnmown when 4 in. high, were larger when seeded with Ladino clover than when seeded alone and given 84 lb. of nitrogen per acre.

According to Lassiter *et al.* (155), the application of manure each year on bluegrass pasture increased the yield of TDN per acre from 13.0 to 22.6% over that of the control pasture. Various manure treatments had little or no effect on the crude protein, but tended to lower the dry matter content of the forage. Van Horn and Dawson (290) stated that the application of manure to permanent pastures increased TDN production 38 to 41%. Eby *et al.* (77) and Nevens (193, 195) also observed that the use of barn manure increased the yield of pastures.

Salter and Yoder (247) concluded that it was more profitable to produce both protein and dry matter by the application of fertilizers to rotating pastures than it was to buy these two materials. Archibald and Nelson (10) also found that intensive fertilization and proper grazing not only increased the yield of dry matter but also increased the feeding value. According to Seath (250), the annual returns per acre over and above the cost of grain fed and fertilizer used over a 4-yr. period averaged $36.73 for unfertilized pastures and $42.84 for fertilized pastures. The best results were secured when the following general procedure was followed in improving a pasture: (a) arrange terraces in pasture to control erosion; (b) apply lime to correct acidity; (c) thoroughly prepare seedbed through plowing, disking, harrowing, and rolling or packing; (d) apply fertilizer, especially phosphorus; (e) seed with adapted clovers and grasses, (f) divide pastures so that grazing can be controlled by rotating cattle from pasture to pasture; (g) control weeds by mowing pastures from two to four times a year; and (h) have water and shade available in all pastures.

PASTURE EVALUATION

Most data available on the amount of milk produced from pasture herbage are given for the entire pasture season. This review, however, is primarily concerned with milk output from summer-grown herbage. Raymond (222) stated that the total annual yield from a pasture is relatively less important than the distribution of the yield between different months of the year. Total annual yield is always dominated by May-June production but, in terms of practical grazing management, herbage production during the other months is of greater

importance. Persistence, palatability, and succulence are more important than total yield. In determining the feeding value of a pasture, the potential level of the grazing animals and not the pasture may be the limiting factor (130). Raymond (222) is of the opinion that new techniques for measuring and comparing pasture outputs should be used over relatively short periods, rather than over the whole season, as has generally been done. Such methods, however, are subject to large errors and are very sensitive to skill in pasture management.

Milk yield. The difficulties in using milk yield as a yardstick in measuring grassland output have been pointed out by Castle (52, 53, 54) and Cox (65). The difficulties include variations in stocking rate, potential milk production of cows, variations in the herbage due to growth and changing weather conditions, and variation in losses due to trampling.

One of the requirements stipulated as basic to high production is efficient harvesting of the pasture herbage (176). Experimental results suggest that the stocking rate is of major importance in this connection. In experiments by Hancock (93), the other factors were kept constant and the stocking rate varied. Thirty cows were used in each of two groups. The low rate stocking group required 30 acres and produced 320 lb. of butterfat per acre, while the high rate group required 18 acres and produced 453 lb. of butterfat. Wallace (296) obtained similar results with two groups of cows. The low rate group required 60 acres of pasture and produced 213 lb. of butterfat per acre, whereas the high rate group required 32 acres and produced 269 lb. of butterfat.

According to McMeekan (179), a stocking rate as high as one milking cow per acre has resulted in an estimated minimum invisible wastage amounting to 35% of all the herbage actually available. Over-stocking may decrease the yield because of close grazing and frequent defoliation, and have long-term adverse effects on the sward.

A review of the early literature dealing with treading (trampling) was made by Edmund (78). Most investigators reported losses of pasture forage due to treading, which altered plant composition and brought about physical and chemical changes in the soil. Increases in treading produced significant and progressive reduction in the yield of all species. Treading loss, when compared with that of mowed crops, amounted to 10 to 15%. Riddet *et al.* (236) of New Zealand attempted to increase milk production from pasture to a level approaching 500 lb. of butterfat per acre. After 5 yr., which included three abnormally dry summers, production had reached only 350 lb. of butterfat per acre. Treading damage may have been the major limiting factor. They suggest that the on-and-off system of grazing might be explored in order to reduce treading. It has been shown that dairy cows can satisfy their appetites in relatively short grazing periods. Then, too, there is a possibility of selecting cows that bring about reduced treading losses.

In a comparison of the nutritive output of a pasture, that the potential level of the grazing animals and not the pasture may have been the limiting factor was suggested by Ivins *et al.* (130). Cox *et al.* (65) used a single cow on each plot, to relate individual pasture intake and milk production. A significant

linear relationship between milk yield and estimated dry matter intake over a 3-wk. period was found.

Composition and digestibility. In the past, most of the methods of estimating pasture herbage intake and its composition and digestibility, and dry matter intake of pasture herbage and estimated nutritive value, were based on the clipping method. The chemical composition, digestibility, and yield of forage crops as feeds for dairy cattle depend on the climate, soil fertility, amount of water available to the plants, botanical composition, and state of maturity of the plants. Some of the early work was reviewed by Huffman (127). Sullivan and Garber (272) reviewed the chemical composition of pasture plants with some reference to dietary needs of grazing animals.

The historical results of Woodman and associates (1926-1938), as compiled by Huffman (127), are shown in Table 3. The effects are shown of increased maturity of both grass and alfalfa on the amount and digestibility of protein and crude fiber. Pasture grass cut every 3 wk. contained 21.1% of protein (dry basis) with a coefficient of digestibility of 79. The feeding value of this pasture was equal to pasture cut at weekly intervals. For all practical purposes, pasture grass cut every 5 wk. was almost as nutritious as the crop cut earlier, and was far superior in productive energy to good meadow hay. Alfalfa cut in prebud contained 25.3% protein and had a coefficient of digestibility of 84. The crude fiber content increased from 22.1% in the prebud stage to 29.7% in the flower stage. The energy value declined from a S.E. (Starch Equivalent) value of 59.8 in the prebud to 42.4 in the flower stage.

Phillips *et al.* (210) studied the changes in chemical composition of timothy tops and found that the percentages of protein decreased, whereas methoxyl, pentosans, crude fiber, lignin, and pectic substances increased with maturity. Waite and Sastry (295) reported that timothy decreased in percentage of protein and increased in fiber with maturity. Armstrong *et al.* (13) observed that crude fiber, lignin, and crude cellulose increased to the seed stage in four different grasses.

According to Laughlin (157), bromegrass harvested at different times throughout the summer in the Matanuska Valley of Alaska showed a decline in protein percentage from 27.4 on May 28 to 4.2 on September 25. Yields of protein were highest in early July, at the bloom stage. Nitrogen application increased the protein content and herbage yields. Heavy nitrogen treatments were more effective when accompanied by phosphorus and potash fertilizers.

Using indicator methods for estimating the amount and composition of grazed forage, Reid *et al.* (228) found that first growth contained 31.0 and 18.8% of crude protein and crude fiber, respectively, on the dry basis, whereas full bloom contained 26.1 and 34.8% protein and fiber, respectively. The grazed herbage contained more protein and less fiber than comparable clipped herbage. Using the same method, Richards and Reid (233) determined the chemical composition of pasture forage, both hand-fed and grazed, in the vegetative, boot-to-early-head, and full-bloom stages of growth. Although the crude protein content and digestibility of both hand-fed and grazed forage decreased with maturity,

TABLE 3

Composition and feeding value of pasture grass and alfalfa cut at different stages of maturity on the dry basis (127)

	Protein	Coefficients of digestibility of protein	Digestible protein	Fiber	Coefficients of digestible fiber	N.F.E.	Coefficients of digestible N.F.E.	Coefficients of digestible organic matter	Starch value
	(%)								
Pasture									
Cut weekly	24.7	81	20.0	15.4	76.7	44.8	81.5	79	67.7
Cut every 2 wk.	23.5	80	18.8	15.9	81.3	44.5	82.8	80	69.8
Cut every 3 wk.	21.1	79	16.7	17.2	79.2	46.7	83.5	80	69.4
Cut every 4 wk.	19.4	76	14.7	21.2	81.7	46.3	82.8	80	67.1
Cut every 5 wk.	18.3	74	13.6	21.9	82.1	47.2	79.1	77	64.7
Good meadow hay	13.8	63	8.7	25.8				65	45.2
Poor meadow hay	7.5	45	3.2	33.5				52	24.0
Alfalfa									
Prebud	25.3	84	21.3	22.1	63.7	38.1	79.8	76	59.8
In bud	20.4	76	15.5	23.9	46.3	42.2	74.2	66	50.8
In flower	17.2	74	13.0	29.7	42.6	39.4	70.2	60	42.4
Average alfalfa	14.2	74	10.5	29.5	50.8	40.9	73.7	....	33.0

the grazed forage contained more protein of greater digestibility. Crude fiber, and cellulose content and digestibilities, decreased with advanced maturity, but the grazed forage was lower in both constituents and higher in digestibilities than the hand-fed herbages. The digestibilities of the NFE and hemicellulose contents of the grazed forage were higher than the hand-fed forage at the various stages of growth. Waite *et al.* (294) measured the output of strip grazing under conditions that largely eliminated the errors suggested by Linehan (160).

The plucking of forage from protected areas until it is similar in appearance to grazed areas was suggested by Sears (248), but allowances for herbage growth during the grazing period are difficult and unsatisfactory.

As has been pointed out, the production of pasture in terms of growth and/or milk production is the best measurement of pasture output. Methods used in pasture studies have been reviewed and recommendations made by a committee on pasture research (204). These include the use of "unit grazing days," TDN output, moving techniques, etc.

Linehan *et al.* (160) showed that over a period of 3 yr. a clipping method gave an output of 101% of that obtained from animal data. During short grazing periods, however, the variation of the measurement of pasture output by mowing techniques and animal output data has not been satisfactory, probably because of selective grazing. Consequently, there is increased interest in methods of determining the amount, chemical composition, and digestibility of grazed herbage.

Indirect methods. Digestibility-indicator methods have shown the most promise. These involve the determination of the indigestibility of the pasture and the fecal output. The indicator for obtaining the indigestibility of grazed herbage should be a naturally occurring substance in the pasture forage, whereas an indigestibile external indicator should be used to measure the fecal output. The combination of the two values should give the total intake.

Two methods are used to measure the indigestibility of herbage: (a) the ratio technique, which requires a naturally occurring indigestible indicator in the forage and the determination of the indicator in both herbage and feces, and (b) the fecal-index technique, in which the indicator is not necessarily indigestible and is measured only in the feces.

Commonly used internal indicators are lignin, methoxyl groups, crude fiber, nitrogen, and plant chromogens. The principal external indicator for measuring fecal output is chromic oxide (Cr_2O_3). Several reviews describing indicator methods are available (140, 223, 227).

Most investigators have found that lignin ratios are unreliable indicators of digestibility. On the other hand, Ellis *et al.* (79) reported that lignin was not digested in any significant amount. Kane *et al.* (141) found complete recovery of lignin and chromic oxide and concluded that they are suitable for digestive studies. Ely *et al.* (80) used the method of Ellis *et al.* (79) and concluded that lignin recoveries were lower than are desirable for an inert tracer material. According to Balch *et al.* (20), crude lignin as determined by the method of Armitage *et al.* (11) showed negligible digestibility and that it should be satis-

factory as the basis of a ratio technique for the dairy cow. It would appear that lignin could be employed as a fecal indicator when improved methods of lignin determination are devised.

Several research workers have used fecal nitrogen or protein in the estimation of forage digestibility. Gallup and Briggs (86) observed that the excretion of nitrogen by steers consuming hay was related to dry matter intake. Raymond (224) and Lancaster (151) also reported that the concentration of nitrogen in the feces of animals was related to the amount of grass consumed. Forbes (83) and Homb and Breirem (124) showed that the amount of nitrogen excreted per unit of dry matter intake was not constant, but was related to the nitrogen content of the forage. As the result of more recent studies, Lancaster (151) modified the method by relating the ratio of feed consumed to the nitrogen content of the feces. His formula may be used to determine both digestibility and forage intake. Raymond *et al.* (224) found that the percentage of organic matter digested by sheep was related to the percentage of nitrogen in the ash-free dry matter of the feces. Milford (180) showed a highly significant positive correlation between dry matter intake and total nitrogen in the feces. Digestibilities of organic matter calculated from fecal nitrogen showed fairly good agreement with values determined by conventional methods for unfertilized grass, but were high for nitrogen-fertilized grass.

A system of utilizing naturally occurring plant chromogen(s) as an internal indicator in pasture evaluation has been worked out by Reid *et al.* (231), who found that digestion coefficients obtained by the chromogen-ratio compared favorably with results obtained from conventional digestion trials. In later studies, Reid *et al.* (230) found that selective grazing and hand-plucked forage samples were not identical with the herbage grazed by the animal and that 100% recovery of ingested chromogen was not obtained when grain was fed in digestion trials with cows. It was noted, however, that there was a relationship between fecal chromogen concentration and forage chromogen concentration (and, hence, digestibility). For successful application, this method did not require sampling the grazed herbage or complete recovery of ingested chromogen.

In further studies, Smith and Reid (263) improved the chromogen technique. Lancaster and Bartrum (152) and Kane and Jacobson (139) found that extracts exposed to strong light or to moderate amounts of light for as long as 6 hr. did not increase the pigment in the fecal extracts. Satisfactory results were obtained when this method was compared with conventional digestion trials (139, 156, 231, 263).

Ingested chromic oxide (Cr_2O_3) was completely recovered from the totally collected feces of several species of animals, and when used in the ratio technique obviated the need for data on the amount of feed consumed and feces voided (227). If Cr_2O_3 passes into the feces in a predictable manner, the total collection of feces could be supplemented by periodic grab samples. Hardison and Reid (98) found that the Cr_2O_3 excretion-time patterns were remarkably similar for steers and cows grazing different pastures during different seasons. Smith and Reid (263) estimated the output of feces equally well when the Cr_2O_3

was administered in capsules or in concentrate feeds. The effectiveness of the procedure for measuring fecal output was the same, regardless of whether Cr_2O_3 was given at 6 A.M. or 4 P.M., or at both times.

Davis *et al.* (69) observed a day-to-day fluctuation in Cr_2O_3 excretion in cattle which was overcome by ten-day collection periods. Putnam *et al.* (217) concluded that the time of Cr_2O_3 administration was of primary importance in respect to the time-concentration relationship of fecal Cr_2O_3, and that so-called physiological, diurnal effects were of little importance. A 12-hr. sampling procedure would be more accurate than unequal fecal sampling times.

To circumvent the use of total fecal collections with grazing animals, Hardison and Reid (98) investigated the possibility of using small, randomly procured fecal grab samples in a limited trial, and found close agreement between the chromogen content of samples taken in the morning and afternoon with those taken from the totally collected feces. Other workers reported large daily variations in chromogen excretion and without a definite pattern. However, fecal grab samples have given results comparable to those obtained by the total collections in several trials, according to Anthony *et al.* (9) and Kane *et al.* (138). From data concerning dry matter intake obtained by the chromic oxide–chromogen technique (98, 231), it can be assumed that grab samples are satisfactory in the estimation of forage digestibility.

APPETITE FOR PASTURE HERBAGE

The economic production of milk is based on the use of high-producing cows, that will consume large amounts of high-quality and high-yielding roughage. The feed capacity or appetite of an animal is the total amount of dry matter consumed when offered as much as it cares to eat (190). Many factors affect the appetite of cattle, such as size, health, inheritance, psychological factors, exercise, and milk production; also, factors inherent in the feed and environment, such as palatability, amount of feed offered, bulk of the ration, balance of the ration, and environment temperatures. Palatability is frequently used to denote appetite. Actually, palatability is tested by the use of the cafeteria method, whereas appetite is measured in the weight of dry matter consumed. There are several reviews on this subject (94, 127, 171, 280). Palatability as an index of nutritive value of roughage has been overemphasized, since it is not always a criterion of dry matter consumption.

Shaw and Atkeson (257) determined the comparative palatability of four cereal pastures with dairy cows. The cows grazed 52% of their time on Balbo rye, 24% on common rye, 18% on winter wheat, and 6% on winter barley. The palatability of a pasture composed of several species of grasses is important. Under such conditions, some species become less palatable than others.

Hancock of New Zealand (94) reviewed the early work on animal behavior studies that apply to dairy cattle. Hancock (95) used lactating identical twin cows and found that an outstanding feature of the grazing behavior was its variability, due to external and internal conditions. Of the external factors, climate

in a temperate zone was relatively unimportant. The quantity and quality of the herbage offered were important in modifying the grazing behavior of dairy cattle. Under adverse pasture conditions there was, in general, an increase of total working time (grazing + ruminating). Grazing time increased with the scarcity and quality of forage, while ruminating time was prolonged by poor-quality grass. The adaptation of grazing habits to new conditions was very rapid. The feed requirement for milk production was the most important internal factor determining grazing time. Hardison *et al.* (96) have also reviewed the recent studies on behavior of dairy cattle. The problem of determining accurately the feed consumed by grazing animals was reviewed by Aamodt *et al.* (2). One of the first reliable studies on pasture grass consumption was that of Helms (105), who reported that two lots of six cows each were tethered on grass within large, measured circles. At the end of the day, the grass left within the circle was cut and weighed. The trial lasted 14 days. The cows consumed about 155 lb. of grass per head daily. Graves *et al.* (89) fed grass clipped from irrigated pastures to four Holstein-Friesian cows for periods of 15 to 55 days. The average consumption of individual cows for the entire period varied from 122 lb. for the smallest cow to 152 lb. for the largest. The results obtained with one cow which was switched back and forth from pasture to grass clippings indicated that cows do not consume as much dry matter while grazing as when fed grass clippings. Woodward (310) concluded that a cow will graze about 6% of the immature grass on one acre of pasture, or 30 to 35 lb. of dry matter daily, depending on its size. Husted-Anderson (129) calculated the dry matter intake of pasture from collected feces. The cows weighed from 1,100 to 1,320 lb., produced from 17.6 to 41.8 lb. of milk daily, and consumed 24.2 to 39.6 lb. of dry matter per cow daily from pasture. The feeding of concentrates caused a significant decrease in dry matter intake from pasture. Night grazing increased pasture dry matter intake 20% over day grazing.

MacLusky (161) found that cows consumed 2.0 to 2.2% of their live weight of pasture dry matter, with an average of about 2.1%. According to Hoskins and Line (125), the daily herbage dry matter intakes of 20.8 to 30.3 per cow, equivalent to 20.8 to 25.4 lb. per 1,000 lb. live weight, were obtained for the daily and four-day folding methods.

According to Cox *et al.* (65), experimentation with grazing, lactating cows involved difficulties associated with the usual variations in milk production, variability of the pasture due to growth, and changing weather conditions. They pointed out that a limiting factor would often be the availability of cows giving sufficient milk at suitable stages of lactation. In this investigation, the possibility of pasture evaluation in terms of milk production was examined, using individually grazed cows on a ryegrass-clover pasture. There was a significant linear relationship between mean milk yield and estimated dry-matter intake over a 3-wk. period. The average mean fresh forage intake for 16 cows was 188 lb. per day. The highest value for a single cow was 233, and the lowest was 125 lb. per day. The mean dry matter intake was 28.2 lb. (22.4 to 32.8 lb.) and the mean FCM yield was 32.0 lb. (19 to 48 lb.) per day.

Using the clipping method, Phillips and Baker (209) reported that cows ate 86 lb. of Ladino clover–orchard grass mixture; 70 lb. of white clover–bluegrass mixture, and 72 lb. of lespedeza-orchardgrass mixture per cow daily. The large amount of pasture dry matter consumed was 45.1 lb. daily, reported by Hill and Lundquist (109). According to Kley (146), the highest dry matter intake was obtained on grass 8 to 9 in. high and yielding about 2,500 lb. per acre. Duckworth and Shirlow (76) concluded that dry intake on pasture depended on the crude fiber content; as the percentage of dry matter increased in the pasture plants, the dry matter intake decreased.

There was a highly significant relation between body weight of growing cattle and dry matter intake, according to Martin *et al.* (166). Dry matter intake increased by 3 lb. daily for each body weight increase of 100 lb., provided sufficient herbage was available to satisfy the appetite.

The effect of the level of milk production and calculated TDN consumed from pasture was discussed by McCullough (171). He also called attention to the relation of pasture dry matter intake to dry matter yield per acre. He cited data from the Netherlands to show that cows consumed 44.0 lb. of dry matter when pasture yielded 2,500 lb. of dry matter, and only 33.0 lb. when the yield was 1,568 lb. per acre. With a yield of 572 lb. of dry matter per acre, the intake was only 15.4 lb. per cow per day.

According to Castle (53), the average daily consumption of ryegrass and cock's-foot herbage dry matter per cow and per 100 lb. of live weight was 32.9 and 2.9 lb. in 1940, 30.7 and 2.9 lb. in 1950, and 26.6 and 2.5 lb. in 1951. Using the chromogen technique, Noller *et al.* (197) reported that three Holstein cows produced 54.9, 40.0, and 37.9 lb. of FCM daily for 84 days and consumed an average of 225 lb. of grass daily, with coefficients of digestibility of 75, 72, and 70%, respectively. In England, it is assumed that cows on high-quality pasture consumed 30 lb. of dry matter daily per 1,000 lb. of live weight (309).

Selective grazing by dairy cattle. In the past, most of the data showing the chemical analysis and digestibility of pasture forage have been based on clipped samples, although it was assumed that animals graze selectively. Attempts have been made to simulate grazing by plucking selected pasture forage to determine the chemical analysis and feeding value (143). The technique developed by Reid and associates (34, 99, 230), for estimating herbage intake and digestibility by grazing animals, has made possible the study of the effect and degree of selective grazing. The earlier work was reviewed by Hardison *et al.* (99). Some of the results of an experiment with growing steers are shown in Table 5. These data show a marked degree of discriminate grazing of the herbages. The forage selected by the grazing animals was higher in crude protein, ether extract, and mineral matter, and lower in crude fiber, than the whole clipped herbage available for consumption. All the constituents of grazed herbage were more digestible than those of the clipped herbage fed to confined steers. The digestibility of dry matter and the amounts of crude protein and ether extract decreased, and the amount of crude fiber increased, as first-growth forage became more mature. Although aftermath herbage contained a high level of crude

TABLE 4

Chemical composition and digestibility of constituents of forage hand-fed and grazed (233)

Constituent	Vegetative stage				Boot-to-early-head stage				Full-bloom stage			
	Hand-fed		Grazed		Hand-fed		Grazed		Hand-fed		Grazed	
	Chemical composition	Digestibility	Chemical composition	Digestibility	Chemical composition	Digestibility	Chemical composition	Digestibility	Chemical composition	Digestibility	Chemical composition	Digestibility
	(%)											
Dry matter	.....	72.9	.....	76.9	.....	66.3	.....	69.4	.....	58.0	.....	58.2
Crude protein	16.1	73.7	18.0	77.8	12.0	66.0	13.6	69.5	10.4	60.8	11.5	61.1
Ether extract	4.8	53.7	5.7	60.8	4.3	56.1	5.1	60.4	3.3	30.3	3.8	30.7
Crude fiber	25.2	73.6	22.8	77.5	27.3	65.0	25.4	68.0	31.8	59.1	29.4	59.4
Nitrogen-free extract	46.4	76.5	46.2	80.3	49.4	69.0	47.8	72.1	49.1	59.9	49.2	60.4
Ash	7.5	58.9	7.3	64.6	6.9	50.5	8.0	63.1	5.4	47.3	6.1	46.0
Lignin	5.8	−11.7	5.2	5.7	6.7	−4.9	6.9	5.3	8.6	−9.0	8.5	8.0
Cellulose	29.9	77.8	30.0	81.3	32.0	68.6	30.4	71.6	33.4	61.0	29.8	61.5
Hemicellulose	17.2	76.5	18.2	80.1	19.2	65.8	17.8	69.0	20.2	61.3	23.7	61.7
Starch	8.2	96.0	8.6	96.0	7.8	96.0	6.9	96.0	7.7	96.0	7.7	96.0
Sugars	5.5	98.9	4.9	99.0	6.8	99.0	4.2	99.1	5.2	99.2	12.4	99.2
Pectins	2.2	87.8	1.6	92.3	1.8	75.1	2.0	77.7	1.1	68.3	1.1	68.7

TABLE 5

Composition and digestibility of herbage selected by grazing steers compared with that of whole herbage cut from the same source (99)

Trial No.	Steer No.	Composition of herbage dry matter					Digestibility				
		Crude protein	Ether extract	Crude fiber	N.F.E.	Ash	Dry matter	Crude protein	Ether extract	Crude fiber	N.F.E.
		(%)									
1949 Forage 1											
1	0	19.5	6.4	21.6	45.5	7.0	78	79	62	78	81
	36	17.4	5.2	23.0	46.7	7.7	77	77	60	77	80
	39	17.0	5.4	23.9	46.6	7.1	76	77	60	77	80
Whole herbage		16.1	4.8	25.2	46.4	7.5	73	74	54	73	77
1950 Forage 1											
1	4	22.9	5.0	26.5	39.1	6.5	75	79	47	76	76
	5	25.0	6.2	25.0	36.9	6.9	75	80	48	77	77
	6	24.7	6.8	27.7	33.4	7.4	76	81	50	78	79
Whole herbage		20.2	4.7	27.9	39.4	7.8	73	78	42	74	74
1950 Forage 2											
1	10	26.0	3.9	24.3	40.8	5.0	72	80	37	70	76
	11	23.4	3.4	25.5	43.4	4.3	70	78	31	67	75
	12	24.7	4.4	22.9	43.0	5.0	75	82	42	72	79
Whole herbage		22.0	3.8	26.9	39.9	7.6	72	80	37	70	77
1951 Forage 2											
1	29	26.0	7.0	18.2	34.8	13.9	68	78	62	56	69
	30	23.1	6.2	23.4	34.2	12.6	65	78	61	52	68
Whole herbage		17.0	3.1	34.9	38.5	8.7	57	73	54	45	63

protein and a low level of crude fiber, it was somewhat less digestible than the first growth of forages of comparable composition. These workers concluded that this investigation demonstrated that the chemical composition of clipped herbage was an unreliable index of the chemical composition of herbage selected by grazing animals, and that the digestibility of clipped herbage was an uncertain value and could be quite misleading in regard to estimating the value of pasture under grazing conditions. On the other hand, Murdock *et al.* (189) used three Holstein heifers and presented data which indicated no apparent decline in digestibility from early spring to aftermath forage.

REQUIREMENTS OF COWS ON PASTURE

The principles that apply to the rationing of dairy cows during the winter can not be applied with the same degree of accuracy to cows grazing summer herbage. Energy is the most important dietary constituent in the dairy cow's ration, because of the large amount needed for maintenance, milk production, and grazing. This subject has recently been reviewed (226, 297). Reid *et al.* (228) used the Cornell and New Zealand data and worked out a formula for calculating the TDN requirement for maintenance for cows on pasture. A 1,000-lb. cow requires 7.9 lb. of TDN for barn maintenance, according to Morrison (186). On pasture, the maintenance requirement is about 40% more, or 3.4 lb. of TDN for a total daily requirement of 11.3 lb. of TDN.

According to Barker *et al.* (22), the energy expended in grazing is affected by a number of variables such as the availability of the grass, grazing management (continuous, rotational, strip), climatic conditions, and the incidence of insects, parasites, and other disturbing factors which would increase the energy requirements or reduce the efficiency of feed conversion. As the energy expended is unavoidable and can not wholly be attributed to the nature of the grass, it is recommended that this item be disregarded, since a uniform allowance would be inappropriate. A 1,000-lb. cow requires 6.5 lb. of S.E. for maintenance and a 2.5 lb. S.E. for each 10 lb. of milk testing 4% fat.

Castle (53) reported that the S.E. required for grazing was either 1, 2, or 3 lb. per cow per day, as judged by the appearance of the animal. When strip grazing, 1 lb. of S.E. was allowed per cow daily. According to Woodman (309), the scientific evidence of the magnitude of the energy expended in grazing is scanty, but it would seem to vary from 1 lb. of S.E. per day on pasture of good quality and dense growth to as much as 3 lb. per day on poor pastures carrying sparse growth herbage.

Good pasture herbage usually contains more digestible protein than is required for liberal milk production. Hardison *et al.* (99) showed that cattle select the parts of plants higher in crude protein which are more digestible than clipped forage. According to Pratt and Davis (212), a grain mixture containing approximately 10% protein is adequate to support an average milk production of 30 lb. of FCM on good pasture. When pasture herbage is mature or dried up, it is advisable to use barn-feeding requirements.

The mineral requirements for growth and milk production used for winter-feeding probably apply to summer-feeding. The requirements and brief discussions of known mineral requirements are presented by the National Research Council (192). In certain areas of the world, cattle may need either phosphorus, cobalt, copper, or iodine as a supplement. Sometimes more than one deficiency may be manifested by cows on pasture. Grass tetany associated with disturbed magnesium metabolism is a spring disease and does not usually occur among summer-grazed cattle.

When pastures are not available, winter-feeding information should be used.

PASTURE SUPPLEMENTS

The best supplement to permanent pasture in areas subject to drought is a good temporary pasture especially adapted to local conditions. In many areas, Sudan grass meets this need. The value of supplemental pastures has been pointed out by many investigators, including O'Neil (200).

The amount of milk produced per day by cows on pasture as the only feed appears variable. The results of McCullough (170) indicate that dairy cows could be maintained in excess of 40 lb. of milk per cow daily on pasture alone, provided the dry matter is at least 70% digestible. According to Castle (55), good leafy pasture herbage can supply all the nutrients necessary for cows yielding 60 lb. of milk a day. Turner (285) gave the same estimation. Experiments by Hill and Lundquist (109) showed that cows produce up to 50 lb. of milk per day on pasture alone. As has been pointed out, high-quality pasture herbage is capable of supplying the needs of good producing cows for the production of about 50 lb. of milk (2 lb. fat). Further experiments are needed to determine the effect on milk production of adding various supplements to such pastures.

In many areas, the mid-summer slump in milk production is a common seasonal occurrence. Efforts to prevent or minimize the reduction in milk production include the use of hay, silage, or concentrates as pasture supplements. Seath *et al.* (252, 253) fed hay to prevent summer slump in milk production. In a preliminary trial, insignificant increases in milk production were secured when 9 lb. of white Dutch clover hay was fed daily. In a second trial, milk production was significantly increased when hay was full-fed on pasture, but it was unprofitable. In Kentucky (7), there was no appreciable increase in milk production or body weight when alfalfa hay was fed free choice to Holstein and Jersey cows on pasture during mid-summer. Seath and Elliott (251) found that feeding either corn silage or an extra amount of a grain mixture to Holstein and Jersey cows aided in preventing the late summer decline (August 19-September 11) in milk production. Since grazing conditions were better-than-average, the benefits were not large enough to be profitable. Seath (249, 251) reported some improvement in the level of milk production when alfalfa silage was fed as a supplement to cows grazing a mixture of Kentucky 31 fescue and Ladino clover. Additional grain fed as a supplement to Kentucky 31 fescue pasture increased milk production, probably because of lack of palatability of the fescue.

According to Seath *et al.* (252, 253), a group of cows on bluegrass–white clover pasture was fed all the alfalfa hay they would eat for 2 hr. twice daily, and another group was on the same pasture without hay. Both groups received grain. The dry matter intake from pasture was estimated by combining the chromogen(s) and chromic oxide techniques. The cows receiving hay consumed 29.6 lb., and those without hay 26.6 lb., per cow daily of total dry matter. The hay supplementation did not increase milk production, but increased the gain in body weight 50 lb. per cow during the experiment.

Cole *et al.* (58) investigated the influence of supplementing bluegrass–white clover pasture during the summer with three groups of cows. Group 1 was a control, Group 2 was supplemented with alfalfa hay, and Group 3 received alfalfa silage as a supplement to pasture. Neither supplement increased dry matter intake; however, the portion of the dry matter received from pasture was reduced 33.9% as a result of hay feeding and 13.1% as a result of silage feeding. The three supplement groups consumed 27.4, 18.1, and 23.8 lb. per cow daily, respectively. The group fed alfalfa silage consumed 4.7 lb. less dry matter as silage than the hay cows. The supplementation with silage or alfalfa hay did not affect milk production or body weights. Monroe (183) reported that hay fed to a group of cows on improved bluegrass pasture increased milk production (10 lb. per cow daily), compared with a group that did not receive hay on pasture.

Nordfeldt and Hansson (198) investigated the effect of adding supplements to dairy cows on pasture with the following results: hay (0.9 feed unit per cow) daily) as a supplement to pasture over a 6-yr. period increased FCM 1.5 lb. daily, compared to a control group with no supplement. At the current prices for feed and milk, the increase in milk production was not economically advantageous. These investigators do not recommend the feeding of supplementary feeds to cows on pasture—up to those producing 33 to 44 lb. of milk daily.

Hancock (93) used identical twins to study three levels of pasture nutrition with three groups of cows. Group 1, one milking cow per acre plus 1 lb. of concentrate for each 5 lb. of milk produced; Group 2, one cow per acre with no concentrates; and Group 3, one cow per 0.6 acre with no concentrates. The groups were rotationally grazed on fresh pasture on a 14-day rotation. The Group 1 cows produced 45% and the Group 2 cows 16% more FCM than the Group 3 cows. Per unit area, however, the Group 3 cows produced 30% more milk than the Group 2 cows. The greater milk yield of the Group 1 cows over the Group 2 cows was attributed mainly to their capacity to maintain a high grass intake along with concentrates, so that the total dry matter intake was maintained at a high level. The Group 2 cows consumed 10% more organic matter than the Group 3 cows, and the Group 1 cows consumed 5% more than the Group 3 cows. The intake of organic matter from grass for the three groups was in the same proportions as the ruminating time, which is a good index of the relative grass intake when cattle are grazed on pasture of similar quality. The results indicate clearly that pasture herbage is not a perfect diet for dairy cattle, since concentrates are capable of giving a substantial increase in milk yields when added to a diet of high-quality pasture. It seems likely that this effect is due mainly to the

fact that dairy cows are capable of consuming some concentrates even when they have satisfied their appetite for pasture herbage, and not to specific constituents or associate effects of the concentrates. If this is true, it is obvious that one of the limiting factors to maximum milk production from good-quality grasslands is the volume of grass which cows can consume. The reasons and remedies for this seem worthy of further investigation. The results also indicate that pasturing dairy cows at a stocking rate as high as one cow per acre may result in an invisible loss of at least one-third of all herbage grown. A higher stocking rate is an obvious way of reducing this wastage.

In the Netherlands, Hart (102) assembled the data from 25 experiments when supplements, usually dried beet-pulp, were fed to dairy cows grazing abundant, good-quality grass. The amount of supplement fed ranged from 2.2 to 11 lb. per cow per day. There was a maximum increase in milk production of 4.4 lb. per cow daily. In one experiment, 8.8 lb. of dried beet slices reduced herbage consumption 19%, while increasing total dry matter intake. Similar small responses to supplementary feeding have been reported by Witt (308) and by MacLusky (162), who found that herbage consumption was reduced on poor, but not on good, quality pasture herbage. Hardison *et al.* (96) found that the feeding of 1 lb. of grain for each 6 lb. of milk resulted in a decrease of about 7.5% in grazing time. Burt (47) found that the feeding of 1 lb. of concentrates to 5 lb. of milk reduced daily grazing time by 1 hr. daily.

The experiments of Riddet and Campbell (236) show that New Zealand pastures do not allow full expression of the productive capacity of dairy cows, but it was emphasized that the use of concentrates to obtain maximum milk production from grazing cows may be uneconomical.

Corbett and Boyne (63) fed dried molasses sugar-beet pulp to dairy cows grazing abundant, intensively managed pastures in two trials (spring and summer). In the spring trial, the cows were fed 8 lb. of beet pulp per cow daily. The cows produced 38 lb. of milk per cow daily, which was 3.7% greater than the control cows. In the summer trial, 10 lb. of beet pulp were fed daily, and the milk yield of the supplemented cows was 11.3% greater than that of the control animals. Live weight was not materially affected. The herbage dry-matter consumption in the spring trial averaged 32.2 and 36.2 lb. per cow daily, for the supplemented and control cows, respectively. In the autumn trial, herbage dry-matter consumption for the supplemented and control cows was 27.0 and 30.6 lb. per cow daily, respectively. They conclude that when abundant good-quality herbage is available, it is uneconomical to feed supplements such as beet-pulp to grazing dairy cattle.

Stoddard *et al.* (270) and Arave *et al.* (8) supplemented irrigated pasture with 1 lb. of grain per cow daily, 1 lb. for each 10 lb. of milk, and 1 lb. for each 5 lb. of FCM milk. Milk production was 26.5, 30.9, and 31.6 lb. per day, respectively, in the first trial, and 37.1, 38.3, and 36.8 lb. per day in the second trial. The authors concluded that grain feeding was not profitable. According to Frens and Bosch (85), cows limited to 80% of the control acreage and fed dried pulp increased slightly in milk production. The concentrate supplementation was not economical.

The amount of pasture dry matter consumed when cows have limited grazing periods was discussed by McCullough (170). Reid *et al.* (229) were of the opinion that cows that grazed 2.3 lb/100 lb. of body weight in addition to 7 lb. of grain daily might have shown an increase in milk production with additional grain supplementation.

According to McCullough (169), feeding 7 lb. of hay or 5 lb. of grain daily reduced grazing time by 10%, ruminating time about 15%, but had no significant effect on milk production. He concluded that rumination time can not be used as an accurate predicator of animal performance. MacLusky (162) reported that the supplementation of pasture with 8 lb. of grain per day produced only 2.25 lb. of additional milk. Corbett (62) reported that the feeding of 8 to 10 lb. of concentrates per day, with various protein contents, to grazing cows failed to give an economical increase in milk production. A grain feeding table for cows on pasture was reported by Morrison (186).

METHODS OF USING PASTURE HERBAGE

The earliest method of using forage crops in summer was by open pastures. Eventually, fences came into use. Controlled grazing by means of tethered cows dates back to very early times. Rotational grazing is a relatively new system of using pastures, although the idea goes back into the sixteenth century (266). It involves dividing the land into two or more small enclosures and grazing each plot in turn. The Hohenheim system of pasture management combined intensive cultural practices with rotational grazing (184, 206). The progress in the use of rotational grazing largely has been due to the work of Riddet (235) of New Zealand and to the introduction of the electric fence (121).

Holmes (116) defined rotational management, whether the grass is grazed or conserved, as rapid grazing or cutting at the leafy stage, followed by a rest period, during which the grass regrows to a nutritious stage and replenishes its root system. According to Jones (137), good management of pasture means treating the plants in such a way as to enable them to provide the maximum quantity of leaves at the particular time the leaves are needed, thus preventing the plants from wasting their energy to produce the stems which no one wants. The art of retaining the plant in the leafy condition in June is to nip the little rudimentary seed heads in May that are destined to form the stem in June. Comparison between strains of grass and different methods of conservation needs to be done by reference to the animal itself; the actual production of meat and of milk per acre, rather than on S.E. and protein equivalent.

Rotational pastures. This subject has been reviewed by Holmes *et al.* (122) and by Shepherd *et al.* (258). According to Hodgson *et al.* (110), pasture yields, calculated as TDN, averaged 5,986 lb. per acre for a rotationally grazed pasture and 5,499 lb. for a similar pasture grazed continuously. This was an increase of 8.9% in favor of rotational grazing, but did not take into account the possibility that the forage from the rotational pasture was of higher biological value. Holdaway and Pratt (115), using bluegrass pasture, found no significant difference in TDN yield between rotational and continuous grazing.

Nevens (194), using heifers and milking cows, concluded that rotational grazing increased the yield and feeding value of pastures and also lengthened the pasture season. Nevens pointed out that pasture crops for dairy cattle should be rated on palatability, economy of production, timeliness of yield, length of pasture season, resistance to drought, amount of weeds, trampling, likelihood of bloat or other trouble, and adaptability to climate, as well as feeding value.

Brundage and Petersen (42) used four sets of identical dairy twins to compare rotational grazing with continuous grazing pasture management when no supplemental feed was fed. There was little difference in total or weekly animal production, in the form of growth, milk production, and maintenance from either management system. The area grazed under daily rotational system yielded nearly three times the production per acre as did that under continuous system. The authors concluded that when adequate good pasture and optimum weather conditions prevailed, daily rotational grazing did not seem to increase animal productivity over that obtained with continuous grazing, but did definitely increase the number of animals which could be maintained on a given area. Attention was directed to the necessity of close observation of both pasture and the animals, to insure adequate intake with minimum wastage in a system of daily rotational grazing.

In a comparison of continuous with rotational grazing, Harrison *et al.* (101) reported that the cows on the continuously grazed area produced more milk and consumed more grain than did those on the rotational area. The large amount of grain may have masked any difference between the two systems. Using the rotational system of pasturing heavy milk producing cows, followed by low producers, and then dry cows and young stock, Montague (184) concluded that proper management of the herd and pasture plots, combined with fertilizer treatment, is essential for the greatest net returns per acre in an intensive grassland system. McMeekan of New Zealand (176, 177) gave the following requirements essential to achieving high animal production from grassland: (a) A large amount of high-quality feed must be grown, the seasonal distribution must fit the animal requirement; (b) a large proportion of this herbage must be harvested by the animal, and (c) efficiency of conversion within the animal must be at a high level. The studies at Ruakura, New Zealand, fell into three main lines: (a) same animals, same stocking rates, but different grazing methods; (b) different animals, same stocking rate, and same grazing methods; and (c) same animals, different stocking rates, and same grazing methods. It will be noted that these types cover the three major grazing management factors under farm control.

The experiments at Ruakura were conducted on pastures yielding 8,000 to 12,000 lb. of dry matter per acre, virtually all-year pasture. A 10-yr. comparison of continuous grazing and rotation grazing with two herds of dairy cows was made. The only feed used was hay, for a 2-wk. period. The cows were milked at the same dairy with the same milkers. No cultivated crops were grown and no concentrates were fed. The cows on continuous grazing produced 266 lb. of butterfat per cow and 214 lb. per acre, whereas the cows on the rotational

grazing produced 324 lb. of butterfat per cow and 242 lb. per acre, annually. McMeekan speculated as to the possible reason for the small average difference in butterfat per acre, because of many pasture yield studies that have shown a marked superiority of rotational over continuous grazing in the amount of forage grown.

An experiment conducted to compare milk production of cows fastened in daily strips as opposed to intensive continuous grazing (187) resulted in no production differences. The importance of using animals capable of high milk production on continuous grazing was shown by the work of Wallace (296). The results of using cows of different levels of butterfat production on relative efficiency are shown in Table 6.

TABLE 6

Level of butterfat production and relative efficiency (296)

Butterfat per cow[a]	Butterfat per acre[b]	Efficiency
(lb.)		*(ratio)*
200	182	100
300	248	136
400	302	166

[a] Cows averaging 850 lb. of live weight.
[b] 10,000-lb. utilized dry matter per acre.

Van Horn *et al.* (288) reported data collected over a 5-yr. period with a forage mixture of orchardgrass, alfalfa, and Ladino clover under two systems of management: rotational grazing all season, and rotational grazing after the removal of one cutting for hay or silage. Rotational grazing which started after the removal of one cutting produced an average of 41% more calculated digestible nutrients, including both grazing and harvested forage, than continuous grazing all season.

Pratt and Davis (213), using brome-alfalfa-Ladino meadows, compared continuous and rotational grazing for three pasture seasons in Ohio. The cows were fed grain and hay in the barn. The rotational system consisted in moving the cows from one pasture to another. The pasture was then mowed and ensiled. The continuously grazed pasture remained unclipped. Under drought conditions, the accumulated forage from the rotational meadows was fed to maintain milk production. There was a 20% greater return of milk per acre from rotational grazing. In a Nevada experiment (104), cows were changed from an all-hay ration to pasture (3 to 4 wk.), then back to all-hay. Milk production (FCM) was increased 3.2 lb. per cow per day on pasture. When the cows were given hay as a supplement to pasture, milk production increased 3.7 lb. daily. McMeekan (178) reported very high yields of butterfat per acre from pastures using an intense system of rotational grazing, in which the cows were rotated among pastures several times a day.

Wilbur and Lundquist (304) reported that relatively high milk production may be maintained on good-to-excellent rotation pastures without supplementary feeds.

Lassiter *et al.* (156) summarized the results obtained with dairy heifers for a 13-yr. investigation to evaluate the continuous vs. the rotational system of grazing bluegrass pasture, and found no advantage of rotational grazing under their conditions. The yearly average production of TDN per acre by the continuous grazed pasture was 2,290 lb. and by the rotational grazed pasture, 2,284 lb.

According to Arnold *et al.* (12) and Camping *et al.* (48), a high output per cow can be achieved under a carefully managed, free-grazing system. The validity of the widespread assumption that a rotational system of management is essential for high milk production was questioned.

Strip grazing. Other systems of rotational grazing are called strip grazing, fold grazing, or ration grazing. MacLusky (162) defined fold grazing as a system in which an electric fence is used to allow cattle sufficient grazing for one day at each move. A back fence is used so that the cattle have access to only one part of the pasture at a time. Even without the use of a back fence, fold-grazing in practice improves animal-carrying capacity by enabling the farmer to gauge the grazing requirements of the herd. The surplus is conserved by reducing wastage from fouling and treading (more especially with long herbage), by making the animals graze more of the herbage by slightly reducing the amount of herbage available. This slight restriction of appetite does not impair milk production of cows yielding up to 40 lb. or more daily, provided the herbage offered is leafy and succulent. Fold grazing without close grazing is not an advantage in stock-carrying capacity.

Strip grazing is a general term which includes fold grazing, close grazing, and ration grazing (Holland), where the cows use a strip for a varying number of days. In ration grazing, the strips are grazed either once or twice daily Any of these systems may include the use of a back fence.

According to Holmes (116), strip grazing, when superimposed on rotational management, increases the utilization of herbage so that 20 to 40% more production per acre is obtained. In dry areas, continued strip grazing, three to six times over the same pasture in a season, results in serious fouling and reduced utilization.

The development of intensive pasture management systems has been comparatively recent. The Hohenheim system of pasture management combines intensive cultural practices with rotational grazing (206, 311). Holmes and Waite (121) proposed strip grazing (daily rotational grazing or close folding) with temporary electric fences. This system has been widely adopted in New Zealand and England. Several investigators (71, 102, 119, 215) reported 15 to 40% increases in milk yield from strip grazing as compared with rotational grazing.

The results of a comparison of strip and rotational grazed pastures with milking cows showed no significant differences in milk production (298). A comparison of daily grazing and four-day folding with low-producing cows showed no differences in milk yield, live weight change, or dry matter intake (125). In experiments where cows are fed above their production ability, the results are often misleading.

According to Brundage *et al.* (43–46), the importance of proper pasture management is magnified in Alaska by the brevity of the grazing season, the extended winter feeding period, and the limited amount of cleared land. These conditions emphasize the importance of obtaining maximum utilization of the available land, for increases in dairy farming often may be obtained more easily through an increase in the number of animal units maintained per acre than by an increase in farm size. A comparison was made of rotational grazing and strip grazing on bromegrass pasture. Strip grazing was only slightly more efficient than conventional rotational grazing. In this experiment, however, grain was fed, which may have covered up some of the effects of strip grazing. Arnold and Holmes (12) found no significant difference in milk production between strip and continuous grazing on nitrogen-fertilized pastures, compared with pastures receiving no nitrogen.

Brundage and Sweetman (43, 44) studied the correlation of herbage intake and milk production under rotational and strip grazing by using the combined Cr_2O_3-chromogen procedure to compute apparent dry matter intake and digestibility. Digestibility was a limiting factor under rotational grazing, but not under strip grazing. Shutt *et al.* (261, 262) called attention to the high protein content of immature forage obtained by rotational grazing. Ample and well-distributed rainfall was found to be a most important factor in the successful operation of the close-grazing scheme of pasture management. Svenstrup (273) compared rotational and strip grazing on 12 farms in Jutland and 12 farms in New Zealand, and found higher utilization of pasturage with strip grazing in both areas.

Brundage and Sweetman (45, 46) compared alfalfa–brome grass pasture under restricted grazing, and found that the cows utilized the pasture almost as efficiently as under rotational grazing. It was pointed out, however, that efficiency of utilization is a function of the potential producing ability of the grazing animals and the pasture being grazed. As the level of either or both rises, more intensive grazing may be necessary to maintain a comparable level of efficiency. Proctor *et al.* (215, 216) compared rotational grazing with close folding for milk production, using cows producing about 32 lb. of milk daily with no supplement. Close folding brought about 25% increase in milk production over rotational grazing. Better utilization under close grazing was due to less soiling of herbage. Dry cows can be made to eat the herbage following milking cows. Norman (199) reported the results of a comparison of close, lenient rotational, and continuous grazing with cattle. The resulting swards differed only slightly in agronomic quality. The lack of any advantage from rotational grazing was attributed to low levels of plant nutrients.

Campling *et al.* (48) compared strip grazing with nitrogen fertilizer, continuous grazing with nitrogen fertilizer, and continuous grazing with clover. The cows in the three groups produced about the same amount of milk (about 42 lb. daily) in trials conducted from May 1 to July 1. In the trial conducted between July 22 and September 22, the difference in milk production between the three groups did not appear to be significant at the lower level of milk pro-

duction (about 26 lb.). Arnold *et al.* (12) reported that wastage due to manure fouling for strip grazing and free grazing was 25 and 75%, respectively.

According to de Geus (71) of The Netherlands, ration grazing is an intensive system of utilizing grass. Cows are restricted by means of an electric fence to an area of pasture sufficient for one day's requirement. This necessitates moving the electric fence once a day. However, if the grass is abnormally long, two moves are made in one day. In continuous grazing, losses up to 40% occurred, compared with an average of 24% for ration grazing. At the Wisconsin Ashland Branch Station, the milking herd was divided into two groups: one group was rotationally grazed and the other group strip grazed (changed twice daily). The estimated TDN yields per acre for 1955 were 2,536 and 5,121 lb., and for 1956, 2,087 and 2,489 lb., respectively. The acres per cow for 1955 were 0.70 and 0.35, and for 1956, 1.10 and 0.65 (153).

A pasture was strip grazed and, although no supplemental feed was fed, the heifers gained 1.32 lb. daily, or 755 lb. per acre in body weight. Estimated TDN per acre was 5,227 lb. (165).

Soilage. Greater mechanization has made practical the system of cutting the forage and hauling it to the cattle. The early work on soilage was reviewed by Kildee *et al.* (144). The recent revival of soilage is due to mechanization which has reduced man-labor requirements. The freshly cut material is no longer fed on the ground but in forage racks or wagons with open sides. The terms zero pasture and green chop have been used. Since neither of these terms fits the bill, there is a tendency to revert to the old terms, soiling or soilage.

Graves *et al.* (89) obtained production data from a few cows receiving immature grass as pasture, soilage, hay and silage. The pounds of milk produced per acre were 4,575, 4,041, and 7,944 lb., respectively. The pasture group produced slightly more total milk than the soilage group, but the soilage cows produced 60% more milk per acre, according to Gullickson *et al.* (91). Henderson *et al.* (106) used milking cows to compare soilage with rotational grazing over a period of 3 yr. A grain mixture was fed at the rate of 1 to 8 lb. of milk daily during the 1st and 3rd yr., and 1 to 6 lb. during the 2nd yr., in an effort to prevent losses in milk production and body weight in both groups. The area required for 37 cows on soilage feeding was 32.9 acres as compared to 44.8 acres on pasture. The cows on pasture produced more total milk than the cows on soilage, but this difference was not significant. These results may have been due to the amount of grain fed and low milk-producing ability of the cows in both groups. Rotational grazing and soilage were compared, using six pairs of open heifers. No grain was fed and the results covered three, 2-yr. periods (106). The soilage-fed heifers gained 1.20 lb. and the pasture-fed heifers 1.55 lb. per day. The results appear to be in favor of pasture. Attention was called to the following points: the daily labor requirements for soilage practices will vary among farms because of field locations, crop yields, methods employed in harvesting the soiling crop, and herd size. Heavy rains not only make chopping difficult, but sometimes it is impossible to get necessary equipment into the fields. A field adjoining the barn should be available for grazing when the machinery

breaks down, or when heavy rains or other emergencies occur. There is also the problem of having a series of crops available to insure a constant supply of good-quality forage. Annual crops such as Sudan grass and millets do well in mid-summer, when many regular crops are in a relatively dormant stage due to lack of moisture and/or high temperature. Crops such as alfalfa, clover, brome grass, and orchardgrass that respond rapidly after cutting are essential. A hard-surface feeding platform is also essential to insure good sanitation. A cover or shade should be provided to prevent excessive moisture losses and to maintain palatability of the soilage.

The results of Henderson *et al.* (106) are not in agreement with those of Runcie (243), who found very little difference in the acreage needed per cow by grazing (0.87 acre for strip grazing) and soilage (0.77 acre for soilage). Runcie pointed out, however, that soilage enables the farmer to make better use of forage fields which, through distance or inaccessability, can not be used for grazing. Also, bloat is less likely to occur.

Bateman *et al.* (26, 29) compared pasture and soilage over a 2-yr. period. The results are shown in Table 7. Milk production per acre was about the same for 1957, but in 1958 the pasture cows produced 10,249 lb. and the soilage cows 9,109 lb. of milk per acre. The value above harvest costs in 1958 was $313 for grazing and $185 for soilage. The pasture season was 159 days and the soilage season was 136 days. It is necessary to allow plants to become more mature for soilage than for pasture. These data show the importance of proper grazing management in conducting comparative tests where grazing is involved.

A comparison of ten-day rotational, one-day strip grazing, and soilage was made by Smith and Keyes (265), using pasture forage, mostly grass. There was no significant difference in the amount of TDN produced per acre. The average feed costs of producing 100 lb. of milk by these three systems of management were $1.19, $1.29, and $1.55, respectively. There were no significant differences in the amount of milk per acre by the three systems of management. This may

TABLE 7

Production per acre from two methods of harvesting (26)

	Year	Graze	Green chop	Diff. green chop (+ or −)
Milk production 4% FCM *(lb.)*	1957	8,602	8,601	−1
	1958	10,249	9,109	−1,140
Butterfat production *(lb.)*	1957	349	345	−4
	1958	401	359	−42
Total man-hours	1957	11.8	29.0	+17.2
	1958	14.4	41.8	+27.4
Total machine-hours	1957	8.8	24.0	+15.2
	1958	4.6	30.9	+26.3
Harvest cost *(dollars)*	1957	.....	.....	.....
	1958	27.0	117.0	+90.0
Gross value per acre milk selling at $3.32 per cwt.	1957	286	286	0
	1958	340	302	−38
Gross value above harvest cost *(dollars)*	1957	.....	.....	.....
	1958	313	185	−128

have been due to the level of grain feeding, or to the limited milk-producing potential of the cows, or both.

According to Hoglund (113), an average of 3.03 acres per cow provided pasture and winter forage on farms where soilage was practiced during the grazing season, compared with 3.45 acres for farms where pasture was grazed in the usual way. In Minnesota (91), an average of 0.87 acre was required per cow when forages were green chopped compared with 1.45 acres when grazed. Production of milk was 2,528 lb. per acre for the green chopped forage and 1,525 lb. for the grazed pastures. It was estimated that 25 to 40% of the crop was wasted when pastures were grazed. More waste occurred with some grasses than with others.

Myers (191) visited farms where soilage was practiced, and concluded that green chopping (soilage) is best adapted to conditions where the size of the dairy enterprise is limited by land resources. Soilage may be adapted to some large farms where land is not a factor limiting the size of the dairy enterprise. Alternate uses for crop-land, such as production of high-value crops for sale, may be a more profitable use of resources than pasture and forage for dairy cattle. Myers (191) called attention to the advantages and disadvantages of the soilage system. Some of the advantages were: (a) Greater utilization of forage is possible than with rotation grazing or with continuous grazing, because of less loss from fouling due to droppings; (b) less energy is expended by cows in obtaining feed; and (c) damage to the soil and grasses through compaction is reduced. Some of the disadvantages of soilage were: (a) Additional costs are involved when the pasture crop is cut and brought to the cows; (b) a forage harvester, hauling, and feeding equipment are necessary, which means new capital investment if these implements are not already owned; (c) additional labor and power are required; (d) wet fields may make the daily job of cutting and hauling the feed difficult; and (e) the job may interfere with other work during peak labor periods. The additional costs may be offset by increases in income or by reduction of other costs. An increase in the size of the herd and greater sale of milk are possibilities, in some instances. Unusual conditions may justify soilage on some farms if pastures are located long distances from the barn, water is not available in the pasture, strip grazing makes fencing expensive, or the cost of grazing in the usual way is excessively high.

According to Hoglund (112), dairy farmers can decrease the acreage of pasture crops by as much as 25 to 30% by using soilage, but a farmer should consider the year-long feed requirements when he calculates the economic returns for different pasture systems. The extra forage saved might be used to feed the cows more forage during the pasture or barn-feeding periods, or it might be used to feed more cows. An individual farmer should consider whether he can afford to spend from 30 to 90 min. daily in chopping and hauling pasture crops to his cows. Will his labor earn him more when it is used on other enterprises? A dairy farmer should also consider whether the additional investment needed to shift to a soilage system might earn more money when invested in more or better cows, more fertilizers, or in a number of other alternatives.

By the use of a technique for determining pasture forage intake and digestibility, Reid *et al.* (228) compared alfalfa grazed conventionally with alfalfa fed as a soilage crop. The results in Table 8 show that the cattle, by selective grazing, consumed forage higher in protein and lower in fiber with a higher TDN content.

TABLE 8

Comparison of the composition and digestibility of alfalfa grazed and fed as a soiling crop (228)

Growth stage	Treatment	Composition of dry matter			Digestibility	
		Protein	Crude fiber	TDN	Protein	Crude fiber
				(%)		
Full bloom (1st growth)	Grazed	31.0	18.8	68	82	52
	Soiling	19.2	34.9	55	77	39
Post-bloom (1st growth)	Grazing	26.1	34.8	66	78	56
	Soiling	17.0	38.5	54	73	42
Vegetative (aftermath)	Grazed	27.7	15.3	73	81	57
	Soiling	19.4	27.3	64	77	52

In comparing relative values of soilage and pasture, the advantages and disadvantages should be weighed. The advantages of soilage are (a) no treading losses, (b) less fencing cost, and (c) no extra TDN required for barn maintenance. The advantages of pasture are: (a) increased nutritive value of immature grazed forage over soilage crop, (b) there is a grazing period before pasture plants yield enough to harvest, (c) higher costs of soilage, and (d) loss of fertilizing value of urine and manure when cows are kept in dry lot. Also, in the the comparison of soilage and pasture, the stocking rate is very important.

Stored feeding. Porter and Skaggs (211) used three groups of ten cows each to compare pasture, soilage, and silage. All cows were barn-fed 10 lb. of roughage daily to prevent bloat. No grain was fed below 17 lb. of milk daily for Jerseys and 24 lb. for Holstein cows. Forage yields harvested by machine were 100% greater than when the crop was grazed. The cows that grazed produced more milk for the amount of feed consumed than the cows fed the whole chopped plant, either as soilage or silage. Pasturing with dairy cows can save more than one-half the labor involved in harvesting the crop, even with the most modern machinery. This saving did not take into account the large investment, repair, and upkeep of the machinery, or the skilled labor required to operate the machinery. Machinery breakdown is a big item for ordinary dairy farmers. The results of this experiment clearly show the economy of grazing compared to machine-harvesting. Only where the dairy operation is on a large scale, and pasturing can not be practiced satisfactorily, does it appear that machine-harvested forage crops will be economical.

Owen *et al.* (201) compared rotational grazing, strip grazing, and silage, using Sudan grass with three groups of ten cows each. The average FCM per cow was 28.0, 26.6, and 29.0 lb., respectively, and the acres required by ten cows for 68 days were 6, 4.1, and 3.4, respectively.

Stone *et al.* (271), using milking cows, compared pasture (millet), soilage (millet), and two types of stored feeding (alfalfa hay and alfalfa silage). Grain

was fed with all rations. Milk production from silage and pasture was significantly less than from soilage and alfalfa hay. The silage ration was superior to pasture for milk production. A comparison of the forages for maintaining body weight ranked in the following order: alfalfa hay, silage, soilage and pasture.

Phillips and Baker (209) compared good pasture with barn feeding with three groups of cows. One group received pasture, another group was pastured but fed grain according to the Haecker Standard for milk production, and the other group was barn-fed the usual ration consisting of hay, corn, corn silage, and grain. The pasture cows were rotationally grazed when the forage was 8 to 10 in. high. Yields of pasture were determined by clipping cages. Milk production was the same for all three groups.

Larson *et al.* (153) and Larson (154) reported the results of an experiment at the Wisconsin Marshfield Branch Station, using three groups of cows. One group was fed hay and silage, a second soilage, and a third was strip grazed (changed twice a day). The estimated TDN yields per acre in 1955 were 4,514, 3,025, and 1,088 lb., and in 1956 were 3,519, 4,823, and 2,969 lb., respectively. The acres per cow for 1955 were 0.48, 0.82, and 1.16; and for 1956 were 0.47, 0.53, and 0.80. Larson (154) discussed a comparison of conventional grazing, rotational grazing, strip grazing, green feeding (soilage), and stored feeding based upon 3 yr. of study. Tall-growing forages of a predominantly alfalfa and grass mixture were used. The losses of forage were 60-70, 43, 31, 2, and 11%, respectively. The milk production per acre was greater for both soilage and stored feeding than for the various systems of grazing. According to Larson, the advantages of stored feeding are: (a) forage can be harvested at the same time, at near the same stage of growth, with the smallest amount of field losses, (b) eliminates most fences, (c) prevents summer drop in milk production, (d) no losses of forage due to trampling, (e) permits closer observation of cattle, (f) less bloat, (g) easier to supply water to the herd, and (h) adapted for use with any sized herd. The disadvantages are: (a) need dry lot near the barn, (b) sanitation may become a problem, (c) dairymen are not freed from winter routine during the summer, and (d) topography of an area, or a single farming unit, is such that grazing is the only practical method of using the forage.

The large losses of forage associated with grazing as reported by Gullickson *et al.* (91) and Larson (154) may have been due to improper grazing management, as suggested by Porter and Skaggs (211). Also, the data reported by Bateman (26), as shown in Table 7, indicate that when tall crops (alfalfa in mixture) are properly grazed, milk production per acre is at least equal to soilage. The works of both Porter and Skaggs and Bateman were conducted under irrigation and, as has been pointed out, there is less loss of pasture forage by fouling when plenty of moisture is available.

In comparing the value of pasture with harvested forages, such as soilage, hay and silage, it is well to keep in mind that cattle use the top of the crop and not both ends of the plants while grazing. The investigations of Hardison *et al.* (97) indicate that alfalfa stems are only 87% as digestible as the tops. It is interesting to note that as the trial advanced the stems decreased about 0.6

of a percentage point per day in digestibility. On the other hand, there was no marked decrease in the digestibility of the top part of the forage as the trial advanced. In using soilage and stored forage, it is not only necessary to haul the stems out of the field but is also necessary to haul most of them back. As has been previously pointed out, the manure problem in the use of both silage and stored crops should be considered in deciding which system of forage utilization should be used.

Pasture for young stock. The calf is born with a four-compartment stomach, but only the abomasum is functional. The age at which transition to the ruminant occurs, from the standpoint of the relative size of the rumen and fiber digestion, depends on the diet (24). Given a limited quantity of milk, the calf will eat alternative feeds at a very early age, provided they are palatable. The trend is to feed the calf so that it becomes a ruminant at an early age, in order to take advantage of the microbial digestion and synthesis of high-quality protein and vitamins. Also, as the rumen starts to function, the incidence of calf scours is considerably reduced. Browlee (41) and Warner *et al.* (300) showed that roughage increased rumen volume, and that a diet of milk and concentrates increased greater development of rumen papillae than a diet of milk and grass. This, in turn, gave more development than milk and hay.

The development of the microbial population in the rumen of the very young calf was reviewed by Roy (240). The protozoan population can be established in the rumen, once the pH is stabilized near neutrality. This occurs at about 8 wk. of age. The beneficial action of the tetracyclines in increasing appetite and efficiency of food utilization during the transition to the ruminant may be due, in part, to their effect in raising the pH of the rumen contents, as shown by Mann *et al.* (163). Roy and Balch (241) found that a calf as early as four days of age showed practically normal rumen contractions and attempted to ruminate.

The widespread belief exists that pasture is of little value to the young calf before 6 mo. of age. McMeekan (175) and Roy *et al.* (242) have shown that calves start eating grass from about the seventh day onwards and can be entirely dependent on good-quality pasture grass at 8 wk. of age.

Experiments at the Ruakura Research Station (5) reported the following findings: (a) calves start to ruminate as early as seven days and rarely later than 3 wk.; (b) by 3 mo. of age the calf has a rumen as large as that of an adult cow, in relation to its body weight, and becomes relatively larger at 5 mo.; (c) calves fed grass in addition to milk show better rumen development than milk-fed calves; (d) early-weaned calves (8 wk.) subsequently show more rapid development of the rumen than late-weaned calves (18 wk.) and are able to handle pasture at the same age; (e) after early weaning (8 wk.), calves require 5 to 7 hr. grazing per day on high-quality pasture to obtain sufficient nutrients to grow at a reasonable rate; (f) on poor-quality, fibrous pastures the calves have to graze longer, waste time in searching for suitable herbage, and ruminate longer in an attempt to satisfy their needs; and (g) calves are highly selective grazers and tend to starve themselves on unsuitable pastures.

Several investigators studied the ability of young calves to digest immature pasture grass. Conrad *et al.* (61) conducted two, seven-day digestion trials on 9-wk.-old calves. The calves digested 71.5 to 73.5% of the dry matter and 70.5 to 82.9% of the cellulose. Armstrong *et al.* (14) fed 10- to 12-wk.-old calves fresh grass (preflower) that had been in cold storage, and obtained coefficients of digestibility for organic matter, crude fiber, NFE, and cellulose of 77, 80, 83, and 84, respectively. The crude protein digestibility, however, was only 60%, which is on the low side. Preston *et al.* (214) found that 3-wk.-old calves digest 75% of the dry matter and 84% of the cellulose of young grass. The forage was mostly grasses, with a small amount of clover and weeds. The digestibility trial covered the 3rd through the 10th wk. of age. McArthur (167), using cows and 8- to 10-wk.-old calves, found that the coefficients of digestibility for dry matter and crude fiber were about the same (80% for crude fiber).

Roy *et al.* (242) reported that calves on pasture were fed 450 lb. of whole milk during the first 8 wk. of life. From the 5th wk. on, the amount of milk was gradually reduced and replaced by an equal volume of water, until the milk was entirely eliminated at 8 wk. of age. During the milk-feeding period, dried grass was provided, but little was eaten. In general, calves preferred pasture grass to the artificially dried product. After weaning at 8 wk., the calves were entirely dependent on pasture grass, except that wheat straw was available. During the spring flush of grass, all of the calves were fed a small quantity of the straw each day. The calves grew normally throughout the spring and early summer, but because of the deterioration of the pasture in late summer it was necessary to start supplemental feeding in the middle of August (England). Five to seven pounds of dried grass were fed per calf daily as a supplement. Most of the calves began to eat grass before they were 3 wk. old.

McCullough and Sell (172) pointed out the possible advantages of raising dairy calves on pasture: (a) reduces labor required to clean calf-barn, (b) decreases sickness and death losses due to improperly cleaned calf-barns, and (c) reduces feed cost. In their study, the newborn calves were left with their dam for 48 hr. From the third through the sixth day, the calves were confined to a small pen, where they were tattooed, dehorned, and taught to drink from a nipple pail. On the seventh day, the calves were placed on pasture. Whole milk was fed for 6 wk. at the rate of 10% of birth weight. The calves were rotated on pasture each week to allow forage regrowth. In experiments where calves were fed grain, it was offered as soon as the calf would nibble at it—usually at about 14 days of age. These investigators emphasized the need for high-quality forage at all times. They also recommended keeping the forage in a leafy stage by frequent mowing and top dressing (forage should be kept under 10 in. in height), dividing the pasture area into small lots, and rotating at 14-day intervals. If internal parasites are a problem, place calves on pasture at 1 wk. of age and continue milk feeding for 6 wk. If grain is to be fed, commence grain feeding at 2 wk. of age. Do not attempt to grow calves on poor pasture, but provide shade, water, salt, and adequate feed at all times.

Conrad and Hibbs (60) placed one group of calves on Ladino clover pasture at 13 to 26 wk. of age and compared them with barn-fed calves on a 2:1 ratio of hay to grain. The calves fed Ladino clover pasture and 2 lb. of oats daily gained more rapidly from 18 to 26 wk. than barn-fed calves. The results indicate that good legume pasture may be substituted for hay in the high roughage system after calves are 3 mo. of age.

Stillions *et al.* (269) compared barn feeding, alfalfa-Ladino-brome pasture alone, and the same pasture with hay free choice. The calves were placed on pasture at eight to 14 days of age and limited whole milk was fed to 49 days of age. The calves consumed an average of 64.5, 43.4, and 36.9 lb. of grain, and 44.5, 0.0, and 10.1 lb. of hay, respectively, during the first 60 days. The average daily gains were 0.97, 1.14, and 1.10 lb., respectively. These differences were not significant. From 60 to 116 days of age, the calves consumed an average of 187.3, 110.0, and 107.9 lb. of grain and 256.2, 0.0, and 20.5 lb. of hay, with an average daily gain of 1.46, 1.67, and 1.70 lb., respectively. The differences in gains were not significant. Fecal egg counts were negative. Feeding hay with pasture appeared to improve fecal firmness and the general appearance of the young calves.

The parasite problem is serious in the Southern States. Grinnells (90) stated that calves should not go to pasture until 10 mo. of age. When internal parasites are not a problem, thrifty calves do well on pasture after they are 2 mo. of age if they are supplied with supplementary feed, shade, and fresh water. McCullough (172) found that calves raised by the calf starter method did well when placed on good pasture at seven days of age with no hay. Rust *et al.* (244) placed 3- to 4-mo.-old calves on good bluegrass pasture. One group received 3 lb. of grain per calf per day and gained an average of 1.2 lb. daily through the summer; whereas, the group that received no grain gained 1.0 lb. daily for the same period. The average dry matter intake from pasture was 2.6 lb. per 100 lb. of body weight for the group receiving grain, as compared to 3.7 lb. for the group receiving no grain. The coefficients of digestibility of the dry matter and protein of pasture herbage were 64.5 and 60.6%, respectively. In general, the grain-fed group had a lower level of parasite infection than the no-grain group.

In the past, high-quality forage in the form of pasture was seldom available for heifers and cows. In evaluating the early work, it is well to keep this in mind. Bender and Bartlett (30) observed losses in weight of 40 to 100 lb. per head during the first months after fat 2-yr.-old heifers were turned on pasture; whereas, heifers winter-fed on roughage suffered no loss in weight and made greater total gains for the season. According to Hayden (103), 29 Jersey heifers, 1 to 2 yr. of age, kept on pasture for 159 days, made daily gains for the year equivalent to 0.8 lb. per head; whereas, 22 Holstein heifers of the same age, pastured 151 days, made daily gains during the year amounting to 1.0 lb. per head. Henderson and Anthony (107) reported that daily gains of yearling dairy heifers kept on pasture for 194 to 203 days ranged from 0.3 to 1.13 lb. per head. Heifers kept on pasture as the only feed gained 0.7 lb. for the Brown Swiss and up to 0.96 lb. for Ayrshire heifers daily.

Reid *et al.* (229) used six pastures at three stages of growth to determine the amount of nutrients consumed by growing steers. The results showed that free-grazing steers weighing from 500 to 600 lb. consumed sufficient digestible protein and TDN to meet their needs for growth without supplementation. Severely restricted intakes of protein and/or TDN resulted in reduced gains in body weight.

DISORDERS OF DAIRY CATTLE DURING THE SUMMER

Cows on pasture may be affected with bloat, or poisoned by eating plants that contain toxic levels of hydrocyanic acid.

Bloat. One of the problems arising from using improved pastures as feed for dairy cattle is that of bloat. Normally, cattle have an efficient eructation mechanism and are able to belch several times as much gas as is ordinarily produced. The subject of bloat has been reviewed previously (56, 57, 74, 132, 203); consequently, this review will be limited to some of the recent papers dealing with pasture bloat.

It is difficult to obtain estimates of the loss of dairy cattle due to pasture bloat, since surveys on the incidence of bloat and deaths include all classes of cattle and all kinds of bloat. Nichols (196) reported that a survey of over 8,000 farms in Wisconsin showed that about 30,000 cows bloated each year; about 6,000 died, which represented a loss of more than $2,000,000 in Wisconsin alone. An extensive survey conducted by the New Zealand Dairy Board in 1953 and 1954 (131) indicated that of the 4,943 farmers who provided information, about 12% considered bloat to be a serious problem in their herds. One dairy cow in every 200 died from bloat during that period in New Zealand. This loss, however, does not take into consideration the loss in milk production which is due chiefly to the decrease in feed intake. This was shown in the average dry matter intake recorded for the various degrees of bloat in stall-fed identical twins by Johns (131). Another loss is due to the fear of bloat, which results in growing less productive pastures.

Bloat may originate in the animal or in the herbage but, in either case, the eructation process fails. The gas produced in the reticulo-rumen collects as a pocket in the dorsal rumen at a point higher than the cardia, the entrance to the esophagus. The cardia is normally below the level of the ingesta. Eructation has to be a coordinated process involving activity of the reticulum, rumen, and esophagus.

The cause or causes of bloat. The cause of this malady is not known, but there are many theories which attempt to explain this condition, such as too much gas production, paralysis of the rumen musculature, the physical nature of feed, and foaming or surface tension. These theories have been described by several investigators and will not be discussed in this review.

Pasture management. As was pointed out by Johns (132), the pasture should not be managed so that mature grass is dominant. This procedure may result in decreased nutritive value of the forage. Dougherty (74) pointed out that pastures containing less than 50% legumes may be considered safe. According

to Johns (132), safe proportions will always be difficult to define, since they will depend on the potency of the legume, the appetizing quality of the mixture, and the selective grazing habits of the cattle. Also, the cost of establishing and maintaining legume grass mixtures or straight grass pasture may be high. Troughton (281) produced bloat in grazing trials with pastures of less than 33% clover under New Zealand conditions.

Controlled grazing includes a restriction of legumes, especially the succulent tops which are believed to be the most potent part of the plant, and an alteration in the grazing method to reduce the rate of intake (132). Control of selection has been accomplished by the use of soilage, but this practice does not necessarily insure freedom from bloat.

The elimination of selective grazing by strip grazing to make the cattle consume the whole plant has been suggested. As was pointed out by Reid (225), this method may reduce the incidence of bloat, but can not give full control of severe bloat without reduction of forage intake and a consequent loss of milk production. This system has a higher labor cost and may lead to overgrazing.

Feeding of roughage. The effectiveness of Sudan grass hay and Sudan pasture in the prevention of acute bloat in cattle was reviewed by Cole and associates (57). Johns (131) failed to prevent acute legume bloat in dairy cattle prefed up to 2 lb. of hay per animal. Hogg and Barrentine (111) prefed 5 lb. of hay to cattle before they were grazed on Ladino clover and found a reduction in bloat in the morning but not in the afternoon. Recently, Colvin *et al.* (59) found that the over-night feeding of an average of 12 lb. of oat hay per cow significantly reduced the incidence and severity of acute bloat in dry cows when pastured on succulent, immature alfalfa.

Antifoaming agents. Authorities are not agreed on the relative occurrence of frothy and free gas pasture bloat. In frothy bloat, the degree of bloat appears to be associated with the stability of the foam. In general, antifoaming agents act by displacing the foaming substance from the surface of the foaming liquid and by replacing it with a nonfoaming surface layer. The feeding of methyl silicone (a good defoamer in vitro) as a bloat preventative does not appear to be effective, according to Johns (132) and Hungate *et al.* (128).

The most effective antifoaming agents thus far developed appear to be the emulsified vegetable oils. These may be given as a drench, added to the drinking water, or applied to the pasture as a spray. Emulsified peanut oil is applied at the rate of 2 oz. per cow daily. Bloat was also prevented on clover pasture by spraying with tallow. Whale oil, medicinal paraffin, vegetable turpentine, and a richinoleic acid derivative were effective in the prevention of bloat, but some of these products tainted the milk (173, 174).

The mixing of either soybean oil or lard oil with lecithin greatly reduced the incidence of bloat when mixed with the grain fed to the animals before being turned to pasture (134). Bloat occurred among cattle pastured on land that had been fertilized with phosphate. The addition of 0.25 lb. of soybean oil per feeding in a grain mixture gave good control for several hours, but ground soybeans (oil content approximately 0.25 lb. per feed) failed to reduce bloat.

Lard oil (100–200 ml.), administered by stomach tube, gave prompt relief in severe cases of bloat (39). Johnson *et al.* (134) and Brown *et al.* (39) added 1 to 2% of water-dispersible oil to the drinking water and reduced the incidence and severity of pasture-type bloat.

Reid (225) prefers the use of oil sprayed on the pasture, rather than the addition of oil to drinking water. It is necessary to maintain an effective level of oil in the rumen throughout the time the animal is grazing a dangerous pasture. It was early found that a limiting factor in the case of oils and fats is the rapidity with which they are lost from the rumen by passing down the digestive tract. Good bloat control was achieved by spraying oils on pastures, but this is only an emergency measure. Its greatest values lie in making it possible for cattle to remain in an otherwise dangerous pasture. Antifoaming agents are effective only against frothy bloat. Spraying is laborious.

Penicillin for bloat control. Barrentine *et al.* (23, 24) controlled bloat in steers for one and one-half to three days by feeding 50 to 75 mg. of procaine penicillin or its equivalent as potassium penicillin. They also obtained practical control of bloat by the free-choice feeding of salt, which contained 800 mg. procaine penicillin per pound. According to Johns (133), the use of 100,000 to 200,000 units of penicillin per cow gave good control in the spring (about 50% effective). No penicillin passed into the milk when it was fed. According to Emery *et al.* (81), the incidence of bloat was reduced about two-thirds by feeding 100 mg. procaine penicillin per cow per day, either with the grain or in the salt on a free-choice basis. The efficiency of treatment appeared to decrease as the season progressed. An average incidence of 9.7% bloat, with a mortality of 4.2% of the bloated animals, was found for 739 cows over a 150-day pasture season. Thomas (277) observed no severe bloating in cattle for 12 to 96 hr. after the administration of 100 mg. of procaine penicillin per animal. Control animals showed extensive bloat. Brown *et al.* (39) and Johnson *et al.* (134) confined dairy cattle to a dry lot and fed fresh alfalfa soilage ad libitum. The oral administration of 75 mg. of procaine penicillin per animal daily appeared to reduce bloat when first given to steers. Later in the season, when the bloat-producing potential of the forage increased sharply, there was just as high an incidence of severe bloat in the penicillin-fed group as in the controls.

There is good agreement among investigators that the feeding of penicillin prevents pasture bloat for a variable time after the start of the regime, but its effectiveness becomes less with time. The work of Smith and Emery (264) indicates that penicillin fed to cattle remains in the rumen longer the first day of feeding than after 3 wk. of feeding. These results suggest that a penicillin-destroying principle increases with time and may explain the ineffectiveness of this antibiotic as a bloat preventative with continued administration.

Prussic Acid (HCN) poisoning. The toxicology of cyanogenetic glycosides was reviewed by Kingsbury (145) and Franzke (84). According to vander Walt (287), poisoning of ruminants by cyanogenetic plants depends upon the amount of the plant consumed, the previous diet of the animal, the hydrogen ion concentration of the stomach contents, the percentage of total HCN present

in the free state in the plant, the concentration of cyanide-liberating enzymes present in the plant, and the HCN content of the plant. According to Moran (185), the intact glycoside is not toxic. The toxicity of the compound is caused by hydrolysis which liberates a free HCN molecule. There is little free HCN in healthy, active growing plants (liberated by enzyme). Ruminants are more susceptible to HCN poisoning than are nonruminants. Plants which contain more than 20 mg. % of HCN can be considered toxic. Boyd *et al.* (33) gave the safe limits of cyanide content as shown in Table 9.

TABLE 9

Tolerance to various levels of HCN in forage (33)

HCN	Relative degree of toxicity
(mg/100 g. dry tissue)	
0– 25	Safe to pasture
25– 50	Safe to pasture
50– 75	Doubtful
75–100	Dangerous to pasture
More than 100	Very dangerous

Young Sudan grass (4 in. high) may be high in HCN, but it declines as the plant matures until 16 in. high, when it is safe to pasture. This is the basis for the recommendation not to pasture Sudan grass until belly-high to the cow (33). Contrary to common opinion, frost does not increase the HCN content of Sudan grass. Boyd *et al.* (33) showed that drought did not increase the cyanide content of Sudan grass, which is also contrary to common opinion. The many toxic weeds that sometimes affect cattle have been reviewed by Kingsbury (145).

EFFECT OF PASTURE AND SOILAGE ON MILK FLAVOR

Desirable flavors. In order to continue marketing milk and milk products, it is necessary to maintain and stabilize the flavor that appeals to the consumer. This is of greater importance because of the trend to hold milk longer between the time milk comes from the cow and its consumption. This review will cover the effects of pasture and green feed on the flavor of milk. Several reviews have been concerned with flavors of milk products from a general point of view. Kende (142) concluded that green feed contains some substance or substances which, when fed to the cow, protect milk against certain off-flavors. Since that time, numerous workers have observed the difference in susceptibility of winter and summer milk to oxidized flavor. Brown *et al.* (40) reported that the feeding of dry feed increased the tendency for the oxidized flavor to develop in milk and that fresh pasture decreased this tendency.

Dahle and Palmer (66) found that spontaneous-oxidized flavor of cow's milk was prevented by feeding pasture—green alfalfa and clover. Hening and Dahlberg (108) found that the occurrence of oxidized flavor in milk was much less in the summer months than in winter months, but there was no definite correlation between the exact period of feeding green legumes and the occurrence

of the off-flavor in the milk. Greenbank (88) reported that the development of the oxidized flavor in milk was not always inhibited by feeding green feed. Beneficial effects, however, were indicated by the fact that in every case there was a decrease in the intensity of the oxidized flavor by the end of the 5th wk. on pasture. Garrett *et al.* (87) concluded that alfalfa silage is almost equal to spring pasture in putting yellow color in milk, and is equal to or better than pasture in producing milk of fine flavor and high resistance to oxidized flavor. According to Thurston (278), cows on abundant pasture produce milk that is not susceptible to oxidized flavor, whereas the milk from the same cows on dry feed is susceptible.

Krukovsky *et al.* (149, 150) reviewed the literature, which showed that the carotene, vitamin A, and tocopherol contents of milk fat vary with the type and quality of the roughage fed. A relationship was also found between the tocopherol content of milk fat and the ability of milk to resist reactions that produce oxidized flavors.

According to Krukovsky, the susceptibility of milk to oxidized flavors is affected not only by the antioxidant activity of the fat (tocopherols) but also by the presence and availability of readily oxidizable nonflavor-forming compounds in the plasma phase of the milk. These properties of milk are influenced by the type and quality of roughage fed. Cows that were pastured on predominantly orchardgrass with some Ladino clover, green oats, and alfalfa-brome-Ladino clover produced milk stable to oxidized flavor. Milk from cows pastured on orchard grass was exceptionally stable toward oxidized flavor, whereas milk from cows on alfalfa-bromegrass-Ladino pasture was the least stable of the kinds of pasture tested. Hvidsten *et al.* (126) found that milk produced on pasture is more stable than milk produced in the preceding barn-feeding period.

Undesirable flavors. Good-quality pasture, under certain conditions, may impart undesirable flavors and odors to milk. Babcock (17) found that the feeding of green alfalfa at the rate of 30 lb. per cow 1 hr. before milking produced pronounced off-flavors and odors in the milk. When reduced to 15 lb., the off-flavors and odors were still present. When using alfalfa pasture, the cows should be removed from the pasture 4 or 5 hr. before milking. Roadhouse and Henderson (239) found that feed flavor appeared in the milk 20 min. after the ingestion of alfalfa juice, or following the feeding of green alfalfa or alfalfa hay. These investigators (238) also found that the feeding of green alfalfa or alfalfa hay 1 to 2 hr. before milking produced strong, undesirable feed flavors and odors in the milk. As the intervals between feeding and milking increased, the intensity of the feed flavors decreased. A 5-hr. interval between feeding and milking eliminated the feed flavor in the milk. A distinct and undesirable feed flavor resulted from feeding 5 lb. of alfalfa hay and 10 lb. of green alfalfa 1 to 2 hr. before milking (239).

Davis (70) presented a list of weeds that are sometimes found in pastures and the off-flavors they produce in milk. He also stated that weed taints are naturally more frequent when pasture is scarce. Certain cows may show a desire for certain weeds even when pastures are abundant. Aurand and Moore (16)

reported that three different mineral supplements, advertised to prevent weed flavor in milk, failed to do the job on onion-infested pasture. Wylie (314) reported that the most practical method of onion-flavor control in milk was pasture management.

The feeding of 15 lb. of green rye 1 hr. before milking produced only slightly abnormal flavors in the milk (18). The feeding of 30 lb. of green rye 1 hr. before milking produced a small increase in abnormal flavor. Rye pasture gave more offensive odor than wheat pasture (6). Removing the cows from pasture 4 hr. before milking eliminated or greatly reduced the off-flavors. Trout and Horwood (282) indicate that Balbo-rye pasture does not have the adverse effect on the flavor of milk that is produced by common rye pasture. The methods of avoiding or correcting off-flavors in milk include: careful herd management, feeding an adequate ration, and keeping cows off the weedy pastures 3 to 8 hr. before milking (72).

According to Roadhouse (238), the feeding of 25 lb. of green-cut alfalfa gave a distinct feed flavor to milk, but the off-flavor was most pronounced when the feed was placed before the cows 2 hr. before milking. The effect of grazing cows on an alfalfa-bromegrass pasture was investigated by Trout *et al.* (283). Intense off-flavor was found when the cows were grazing heavily. Alfalfa contributed more to the off-flavor than did the bromegrass. When the cows were kept off the pasture 7 hr. prior to milking, no feed flavors were noticeable in the milk.

Flavor scores showed no clover flavor to be present in the milk produced by cows grazing on a Ladino clover, fescue, and orchardgrass pasture up to 2 hr. previous to milking (259). Abnormal flavor and odor were produced in milk by feeding quantities of rape up to 30 lb. per cow per day 1 hr. before milking (19). When fed immediately after milking, rape had practically no effect on either the flavor or odor of the milk. Milk production was not affected when the dairy herd was taken off pastures for 3 hr. before the afternoon milking (67). Marryatt (164) found that feed flavor in milk was much reduced by keeping the cows in dry lots for 3 hr. before milking.

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SOME EFFECTS OF FEEDING VARIOUS FILLED MILKS TO DAIRY CALVES. I. PHYSICAL CONDITION AND WEIGHT GAINS, WITH SPECIAL REFERENCE TO LOW-FAT RATIONS [1]

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SUMMARY

Various filled milks and low-fat rations were studied in an attempt to determine some of the factors responsible for the unsatisfactory gains, poor physical condition, and deaths which have been reported as occurring in calves fed rations containing highly unsaturated vegetable fat. Under the conditions of the experiment, the results of feeding corn oil filled milk to dairy calves were as follows: (a) animals appeared emaciated and unthrifty, (b) a marked diarrhea was observed, (c) an increased susceptibility to pneumonia and a high mortality rate occurred when infection was not rigidly controlled, (d) symptoms of anorexia and muscular involvement were noted, (e) poor feed utilization and unsatisfactory weight gains were obtained.

Calves fed hydrogenated vegetable oil, lard, or butter oil filled milks and whole milk were not adversely affected in the above manner, except as follows: Animals receiving lard and butter oil, prepared from oxidized butter, also exhibited symptoms of muscular involvement. Supplementation of the corn oil and lard filled milk diets with high levels of tocopherol prevented the development of muscular involvement, but did not improve weight gains of calves fed corn oil. Weight gain and feed utilization data obtained on the various rations suggest that the inclusion of corn oil in the diet depresses growth in some manner.

Diets containing as low as 0.10–0.15% butterfat were not detrimental to growth and physical condition of calves under the experimental conditions. A number of the animals fed several of the filled milk and low-fat rations were satisfactorily maintained on the liquid diets to 5-6 mo. of age.

Previous studies (7, 8, 9, 11, 13) have established that highly unsaturated vegetable oils adversely affect growth and physical condition when used as substitutes for butterfat in the diet of dairy calves. This research represents an attempt to determine some of the factors responsible for the unsatisfactory results obtained with highly unsaturated vegetable oils. Data pertaining to physical condition, appearance and weight gains of calves fed various filled milks and low-fat rations are presented in this paper. Those relating to digestibility, blood, urine, necropsy, electrocardiographic, and other studies will be reported later.

EXPERIMENTAL PROCEDURE

Approximately 150 calves were involved in this study, which extended over a period of 4 yr. The animals used in this experiment were, with several excep-

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tions, purebred or grade Holstein bull calves. All animals received colostrum for from two to three days following birth and were subsequently fed whole milk until they were placed on an experimental ration at approximately 14 days of age. Whenever possible, the calves were maintained on the experimental diets until 90 days of age. Although all trials were not run concurrently, environmental conditions were maintained as nearly uniform as possible for all subjects.

Each calf was weighed at weekly intervals. Frequent observations were made and recorded in regard to the physical condition and other pertinent data concerning each animal. Regarding health supervision, the initial group of calves receiving the corn oil filled milk were treated only when symptoms of respiratory infection were noted, or the subjects were afflicted with infectious scours. All subsequent animals on the corn oil filled milk and other diets were treated with therapeutic doses of penicillin in oil or terramycin in sterile distilled water. These antibiotics were administered intramuscularly, whenever the daily rectal temperature of the calf reached 103.5° F. or greater.

All of the fats and oils studied were refined or fit for human consumption. The filled milks were prepared by homogenizing the various fats and oils into pasteurized skimmilk at a rate calculated to provide a product containing 3.5% fat. Each filled milk was heated to approximately 65° C., then passed twice through a Gaulin two-stage homogenizer at 2,500-lb. pressure. All diets were prepared weekly, except as otherwise noted, and stored at 5° C.

Vitamin A was provided at the rate of 160 I.U. per pound of body weight, whereas vitamin D was fed at the rate of 500 I.U. per 100 lb. of body weight. Both vitamins A (cod liver oil concentrate) and D (Viosterol) were added prior to homogenization. All rations except the whole milk diet were so supplemented with vitamins A and D. Animals fed whole milk were provided only with vitamin D in capsule form. The calves on all diets received the following amounts of trace minerals in elemental form: Fe 70, Cu 7, Mn 10, Co 5, I 1.5, and Zn 0.9 mg. per 100 lb. of body weight. A solution of these minerals was added to the rations. Anhydrous magnesium carbonate was added to each diet immediately prior to feeding at the rate of 5 g. per 100 lb. of body weight daily. A mixture of equal parts of steamed bone meal and salt was fed free-choice.

The whole milk diet consisted of herd milk which was standardized at 3.5% butterfat and subsequently pasteurized and homogenized. The fortified skimmilk ration was prepared by adding sufficient nonfat dried milk solids to pasteurized skimmilk, so as to provide a product which was isocaloric, with the filled milks containing 3.5% fat. This diet contained approximately 0.15% butterfat. The skimmilk ration tested 0.1% butterfat and did not contain any additional nonfat dried milk solids. The fortified skimmilk plus corn oil diet was identical to the fortified skimmilk ration, with the exception that sufficient corn oil was incorporated to provide a product with approximately 3.5% fat. The fortified skimmilk plus hydrogenated vegetable oil ration was prepared in the same manner, except that a blend of hydrogenated cottonseed and soybean oils was used as the source of fat. This type of blend is frequently used in frozen desserts.

A mixture of B-complex vitamins and liver residue was administered orally to provide the various ingredients at the rates indicated in Table 1. As detailed

TABLE 1
B-complex and liver residue supplementation

Ingredient	Mg/100 lb. body weight daily
Thiamine chloride	3.00
Riboflavin	6.50
Pyridoxine	3.00
Calcium pantothenate	15.00
Inositol	100.00
Choline chloride	1,000.00
Menadione	1.07
Nicotinic Acid	15.00
p-Amino Benzoic Acid	10.00
Folic Acid	5.00
Biotin	0.05
B_{12}—0.1% trituration	0.05
Liver residue (20%)	20.00

in a later report, several methods of tocopherol administration as well as tocopherol supplements were investigated. In each instance an equivalent of 500 mg. of DL alpha tocopherol or alpha tocopheryl acetate was administered daily to each calf. While only certain groups of corn oil and lard filled milk animals received tocopherol supplementation, all calves on the skimmilk and fortified skimmilk plus corn oil rations received such supplementation.

The filled milks or other diets as supplemented with mineral and vitamins constituted the sole source of nutrients, except as noted otherwise. With the exception of the skimmilk and the fortified skimmilk plus added fat diets, all rations were fed at levels calculated to provide total digestible nutrients according to the recommendations of the National Research Council Committee on Animal Nutrition (12).

RESULTS AND DISCUSSION

A description and an enumeration of the various diets studied in this phase of the research may be found in Tables 2 and 3, respectively.

TABLE 2
Major components and estimated TDN content of diets

Diet	Lb. skimmilk	Lb. added nonfat dried milk solids	Lb. added fat	Estimated TDN content[a]
	(per 100 lb.)			
Skimmilk	100.0	....	....	8.7
Corn oil filled milk	96.5	....	3.5	15.5
Hydrogenated corn oil filled milk	96.5	....	3.5	15.5
Lard filled milk	96.5	....	3.5	15.5
Hydrogenated cottonseed and soybean oil blend filled milk	96.5	....	3.5	15.5
Whole milk	.....	....	....	16.3
Butter oil filled milk	96.5	....	3.5	15.5
Fortified skimmilk	90.5	9.5	....	15.5
Fortified skimmilk plus corn oil	87.3	9.2	3.5	22.0
Fortified skimmilk plus hydrogenated cottonseed and soybean oil blend	87.3	9.2	3.5	22.0

[a] TDN content estimated using digestion coefficients for skimmilk and nonfat dried solids as listed in the 22nd edition of Morrison's *Feeds and Feeding*, and assuming a digestion coefficient of 90 for each added fat.

TABLE 3

Weight gains and efficiency of feed utilization among the various groups for a 76-day experimental period

Group	Diet	No. calves	Av. total gain	Av. daily gain	Av. digestible dry matter intake	Av. gain per lb. digestible dry matter
			(lb.)			
I	Skimmilk	3	67.0	0.88	101.5	0.66
II	Corn oil filled milk (before change in infection control)	5[a]	42.0	0.55	113.5	0.37
III	Corn oil filled milk (after change in infection control)	6	61.0	0.80	122.0	0.50
IV	Corn oil filled milk with intramuscular tocopherol supplementation	4	39.5	0.52	101.3	0.39
V	Corn oil filled milk with oral tocopherol supplementation	6	60.0	0.79	115.4	0.52
VI	Hydrogenated corn oil filled milk	4	110.0	1.45	146.7	0.75
VII	Lard filled milk	6	101.0	1.33	148.5	0.68
VIII	Hydrogenated cottonseed and soybean oil blend filled milk	4	126.0	1.66	175.0	0.72
IX	Whole milk	4	126.0	1.66	165.8	0.76
X	Butter oil filled milk	3	152.0	2.00	192.4	0.79
XI	Fortified skimmilk	4	146.0	1.92	239.3	0.61
XII	Fortified skimmilk plus corn oil	4	128.0	1.68	220.7	0.58
XIII	Fortified skimmilk plus hydrogenated cottonseed and soybean oil blend	2	211.0	2.78	293.1	0.72

[a] Only five of the 12 calves in this group completed the experimental period.

Debilitation, infection, and mortality. Considerable differences were noted in the character and amounts of feces voided by calves on the various rations. All calves on the corn oil filled milks, with and without tocopherol supplementation, were afflicted with a marked diarrhea throughout the experimental period. A marked diarrhea persisted in animals fed the fortified skimmilk plus corn oil ration also. The feces voided by calves in Groups I, XI, and XIII varied diurnally, from firm to markedly loose. In contrast, the feces of animals in Groups VI through X were firm throughout the experiment.

Calves in all corn oil filled milk groups were unthrifty and emaciated. Those in the skimmilk group also were somewhat unthrifty. Although the skimmilk animals did not seem emaciated, they did appear stunted. All calves on the remaining diets studied were thrifty and in good flesh. Subjects fed the fortified skimmilk plus hydrogenated vegetable oil ration, however, appeared extremely fat.

Various calves in Groups II, III, and VII developed some or all of the following symptoms: anorexia, general weakness, unsteady gait, standing with arched back, open shoulders, or prominent suprascapula, stiffness in the fore and/or rear quarters, muscle tremors, difficulty in swallowing and inability to stand. These symptoms usually appeared after approximately 55 days of experimental feeding. Fifty per cent of the animals in the corn oil filled milk groups were so afflicted, while only one of six calves on the lard filled milk diet exhibited such symptoms. In addition, one of the three subjects fed butter oil filled milk developed an acute case of muscular involvement. It should be added that the skin of several corn oil filled milk animals seemed leathery, and the underlying muscles appeared "petrified" during the terminal stages of the syndrome. Thus, it was nearly impossible to reach the jugular vein, even with the sharpest of bleeding needles. None of the animals on the other diets was afflicted with any of the symptoms mentioned.

More than 50% of the calves receiving corn oil filled milk before the change in infection control contracted pneumonia or other respiratory infections. This is in sharp contrast to no cases in a similar group following the change in infection control. Single cases occurred also in the lard and corn oil filled milk with intramuscular tocopherol supplementation groups, as well as on the fortified skimmilk plus corn oil ration. None of the subjects on the other diets contracted pneumonia or other respiratory disorders. Rectal temperatures of 103.5° F. or above were frequently encountered in all groups receiving corn oil or lard filled milk. High rectal temperatures were almost nonexistent in all other groups.

Some animals in certain groups died and others were sacrificed when death was expected momentarily, to ensure accurate post-mortem findings. Prior to the change in infection control, 90% of the corn oil filled milk calves died. Most of these losses occurred during the 76-day experimental period. Several deaths, however, occurred following 12-28 days of additional feeding. Following the change in infection control, only two of six animals in a subsequent corn oil filled milk group died or were sacrificed during the experimental period. One of three calves in the butter oil filled milk group was afflicted with acute muscular involvement and was sacrificed after 89 days of experimental feeding.

In some cases, the subjects were maintained on the experimental rations to 5-6 mo. of age, to determine if any deleterious effects might be manifested as the result of a longer feeding period. No adverse effects were noted, however, when certain calves on the hydrogenated corn oil, hydrogenated cottonseed, and soybean oil blend and lard filled milk diets remained on those rations to 5-6 mo. of age. This was also true in the case of the whole milk and fortified skimmilk diets. In addition, two animals in the butter oil filled milk group were carried to 5 and 6 mo., respectively, despite the earlier loss of one calf on this diet.

The observations concerning the degree of diarrhea, general appearance, and flesh of the experimental calves closely agree with those previously reported by others (7, 8, 9, 11, 13). The symptoms noted in the present study are also strikingly similar to those observed in calves afflicted with nutritional muscular dystrophy (2, 14).

The greater incidence of pneumonia in the corn oil filled milk group suggests an increased susceptibility to respiratory infection in calves receiving this diet. Blaxter, Watts, and Wood (2) have suggested that the high incidence of pneumonia in dystrophic calves may be related to food entering the lungs due to poor control of throat and tongue muscles. A portion of the feed refusals noted in the terminal stages of the syndrome in the present study also might be attributed to an involvement of throat and tongue muscles. The effectiveness of the infection control program used with a later group of corn oil filled milk animals, as well as with all other groups, is indicated by the relatively few cases of pneumonia which occurred following its adoption. The marked reduction in mortality, which resulted after the change in infection control, strongly suggests that infection may have been responsible for a large number of the deaths in the initial corn oil filled milk group.

The finding that calves receiving several of the diets were able to remain on the experimental rations to 5-6 mo. of age without adverse effects is not entirely in agreement with a report by Thomas and Okamoto (15). These workers have found that animals fed whole milk exclusively died when 5 mo. to 1 yr. of age. It should be pointed out that while only one death occurred in three calves fed butter oil filled milk, one of the remaining subjects exhibited muscular lesions upon necropsy. The possible cause for the unsatisfactory results obtained with the butter oil filled milk will be discussed further, in later reports.

Weight gains and feed utilization. The presentation of these data has been complicated by the differences in dry matter digestibility and intake, as well as other factors. Certain diets were extremely low or high in total solids or estimated total digestible nutrient content for experimental reasons. A summary of the weight gain and feed utilization data may be found in Table 3.

The gains made by calves fed the various corn oil filled milks were considerably less than those of Groups VI through X, inclusively. The average gain for the skimmilk fed animals was somewhat greater than that for the groups fed corn oil filled milk, despite a lower digestible dry matter intake by the subjects receiving skimmilk. Gains made by calves in Group XII did not exceed those made by subjects in Group XI, and were substantially less than the gains of Group

XIII. These findings suggest that the feeding of a corn oil filled milk to dairy calves results in a depression of growth and/or poor utilization of dietary fat. The poor gains made by the corn oil filled milk animals are in general agreement with results obtained in earlier experiments using corn, cottonseed, and soybean oils (7, 8, 9, 11, 13).

It is of considerable interest to note that satisfactory gains as well as physical condition were maintained in calves fed the fortified skimmilk ration to 5-6 mo. of age. This diet contained only 0.15% fat. Also, no symptoms of a fat deficiency were observed in calves raised to 90 days of age on the skimmilk diet containing 0.10% fat. These results suggest that a fat content of 0.10–0.15% in an otherwise adequate ration may be sufficient to prevent the development of a fat deficiency in calves as described by other workers (4, 10). It should be emphasized, however, that all animals in this investigation received colostrum and were fed whole milk to approximately 14 days of age. Evidence similar to that obtained with the low-fat rations in this study has recently been reported by Wing, Jacobson, and Allen (16).

An analysis of variance of the feed utilization data revealed that significant differences existed among diets. Further analyses were conducted according to procedures outlined by Duncan (5, 6), to test the differences among various treatment means at the 5% level of significance only. The average gains per pound of digestible dry matter made by the butter oil filled milk and whole milk groups were significantly greater than those of animals fed corn oil filled milks or the fortified skimmilk with corn oil ration. Significant differences in feed utilization were also found among the groups fed hydrogenated vegetable oil filled milks and those receiving corn oil filled milks. In addition, feed utilization by skimmilk and lard filled milk calves was significantly greater than that found on all corn oil filled milk diets, except the corn oil filled milk group receiving oral tocopherol supplementation. It was also established that no significant differences in feed utilization existed among Groups I, VI-XI (inclusive), and XIII.

Apparently the change in infection control beneficially affected gains made by corn oil filled milk calves. It did not, however, significantly improve the efficiency of feed utilization. Tocopherol supplementation of the corn oil filled milk diet also did not improve weight gains or the efficiency of feed utilization, in spite of the absence of muscular involvement. This finding tends to substantiate recent reports (3, 14), in which weight gains of calves afflicted with nutritional muscular dystrophy were not adversely affected. The intramuscular administration of tocopherol resulted in markedly reduced weight gains. This reduction in gain undoubtedly is associated with the mode of supplementation rather than the tocopherol per se. Calves in this group had a greater incidence of high rectal temperatures than the other corn oil filled milk groups.

The growth rate of animals on the fortified skimmilk plus hydrogenated cottonseed and soybean oil blend diet was indeed remarkable. The average daily gain made by the subjects in this group was 2.78 lb., as compared to 2.00 and 1.92 lb. for calves fed the butter oil filled milk and fortified skimmilk

rations, respectively. The weight gains made by the animals in the fortified skimmilk groups suggest that a detailed study of similar diets might of a value from the standpoint of producing choice veal.

Supplementation studies. In addition to the vitamin and mineral supplements already mentioned, several other supplements were fed to additional calves, to determine the extent to which they might alleviate the deleterious effects noted in animals receiving corn oil filled milk. Four calves on a corn oil filled milk ration were fed alfalfa hay throughout the experimental period. These animals were on experiment before the change in infection control was initiated. None of the subjects in this group, however, was afflicted with pneumonia or other respiratory disorders. The feces of these calves were normal in color and varied from loose to reasonably firm in consistency. Two of the animals completed the experimental period of 76 days, and were subsequently sold.

After approximately 66 days of experimental feeding, the physical condition of the remaining pair of calves deteriorated to the extent that it was necessary to remove them from the diet. The average daily gain of the entire hay-supplemented group was 0.74 lb., as compared to 0.55 lb. for comparable subjects fed corn oil filled milk alone. However, the average daily gain for the calves completing the experimental period was 0.85 lb. Two calves which completed the feeding trial consumed an average of 1.9 lb. of hay daily, as compared to 0.5 lb. for those unable to complete the experimental period. Apparently, the consumption of appreciable quantities of alfalfa hay partially alleviated the deleterious effects of corn oil feeding in this study. This indication is not entirely in agreement with the results obtained by others (1), who found that the deleterious effects of feeding a highly unsaturated vegetable oil could not be overcome by the inclusion of alfalfa hay and grain in a ration for dairy calves.

Sodium acetate was administered orally to four calves which were in poor physical condition as the result of corn oil filled milk feeding. In all instances, 21.3 g. of sodium acetate were dissolved in each of the morning and evening allowances of the diet. An immediate improvement in appetite was noted in two of the animals. In addition, a slight increase in daily weight grains occurred in three of the four subjects. These improvements, however, were transitory in all calves, as their condition again deteriorated following approximately 30 days of sodium acetate supplementation.

An additional group of corn oil filled milk calves was supplemented with soya phosphatides, added to the filled milk prior to homogenization at a rate to provide a liquid product containing 0.1% soya phosphatides. This level gave the animals a minimal intake of approximately 5 g. of this supplement per head daily. No noticeable improvement in the condition of the subjects resulted from soya phosphatide supplementation throughout the experimental period.

Streptomycin was administered orally to an additional group of animals on the corn oil filled milk ration. A solution of streptomycin in sterile distilled water was added to the filled milk immediately before feeding, at a rate to provide 45 mg. of streptomycin per pound of milk fed. Such supplementation did not reduce the severity of the diarrhea or have any other beneficial effects upon

the well-being of the calves. Similarly, no improvement in physical condition or weight gains was observed in two corn oil filled milk animals supplemented with high levels of ascorbic acid, in an attempt to counteract the possible toxic effects of corn oil feeding. The ascorbic acid was administered orally, so as to provide 3.6 g. per kilogram of fat fed throughout the experimental period.

It should be pointed out that nine additional calves on the corn oil filled milk diet were removed from the experimental ration at a time when they were exhibiting the typical symptoms already mentioned. A ration consisting of whole milk, alfalfa hay, and a grain mixture was then introduced. The improvement in the condition of six of the animals was almost immediate and very striking. Three of the subjects, however, showed no response to this change in diet. Most calves regained their strength within 5–11 days, and the color and consistency of the feces usually returned to normal within 1–2 wk. The daily weight gains made by the animals doubled or, in some instances, tripled within 2 wk. following the change in regime. Complete recovery usually resulted in 20–40 days. The recovery of corn oil filled milk animals following a change to a ration of whole milk, hay, and concentrates appeared to be dependent upon the extent of muscular involvement and the presence or absence of pneumonia.

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SOME EFFECTS OF FEEDING VARIOUS FILLED MILKS TO DAIRY CALVES. II. FECAL CHARACTERISTICS AND DIGESTIBILITY DATA[1]

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SUMMARY

Young dairy calves were fed low-fat rations or filled milks containing various fats. One group received a corn oil filled milk which was prepared daily rather than weekly, to study effects of storage. All other diets were prepared once each week. Differences in the color of feces were observed among the various rations. Abnormally large amounts of feces were voided by animals fed the corn oil filled milk prepared weekly. Daily preparation of the corn oil filled milk diet or the feeding of hydrogenated corn oil greatly reduced the amounts of feces excreted.

Apparent dry matter digestibility for the weekly prepared corn oil filled milk group was significantly less than that obtained for any of the other diets. The apparent metabolic fecal fat excretion by calves fed a skimmilk diet containing 0.1% butterfat amounted to 5.36% of the fecal dry matter. It was established that the apparent digestion coefficients for butter oil were significantly greater than those for corn oil, hydrogenated corn oil, or lard. Daily preparation of the corn oil filled milk significantly increased dry matter digestibility, but did not significantly increase fat digestibility over the weekly preparation of this ration. Partition of the fecal fat revealed that a considerable portion was excreted as soaps by animals receiving the various diets. The fecal fat partition of calves fed butter oil filled milk was similar to that of apparent metabolic fecal fat, as excreted by animals in the skimmilk group.

The effects of various filled milks upon the physical condition, appearance, and growth of calves have been presented in an earlier paper (1). The research reported here represents a further attempt to ascertain some of the factors responsible for the unsatisfactory weight gains and poor feed utilization encountered when highly unsaturated vegetable oils are fed to dairy calves.

EXPERIMENTAL PROCEDURE

A number of calves from several of the groups mentioned in an earlier paper were used in the digestibility studies reported here. Details on the feeding and management of the subjects were presented in a previous report (1).

Since the animals from several of the groups were highly susceptible to respiratory disorders and often in a weakened condition, the customary collection period used in digestion trials was not employed. Instead, the calves were periodically placed in metabolism stalls for a three-day period at approximately 20-day intervals, beginning at 35 days of age. Total collection of the feces voided by the subjects was made during the 48 hr. following a preliminary

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period of 24 hr. Feed intake was held constant for approximately 1 wk. prior to the collection period. This manner of collection did not unduly jeopardize the health of the individuals and prevented interference with other phases of the experiment, particularly those dealing with the development of muscular involvement. It also afforded an opportunity to determine whether digestibility was progressively affected during the experimental period. Following the weighing of the feces voided during the collection period, representative samples were taken and stored at −20° C. until analyses were performed. The storage period for the fecal samples did not exceed 8 wk.

Dry matter was determined by oven drying. A sample of the dried feces was pulverized and used in the determination of total fat content and the various fat components. A slight modification in the method of Van der Kammer and associates (12) made it possible to ascertain the grams of soaps as well as free fatty acids and neutral fat present per 100 g. of fecal dry matter. The original method gives values for neutral fat and the total fatty acids present as fecal soaps and free fatty acids. In the modification an additional titration was performed on a portion of the dried fecal sample, which was refluxed without the addition of HCl to avoid the liberation of the fatty acids present as soaps. The value obtained from this additional titration was taken as the original free fatty acids present in the fecal sample. The difference between this value and the one obtained after refluxing with acid was taken as the original soaps present in the sample of dried feces.

RESULTS AND DISCUSSION

Fecal characteristics. As mentioned in the earlier report (1), marked differences in the consistency of the feces were found among the various dietary regimes. The mean daily fecal excretion values in Table 1 provide a reasonable

TABLE 1

Fecal output by calves on the various diets

Group	Diet	No. calves	No. trials	Mean dry matter intake	Mean daily fecal excretion [a]	Standard error of mean
				(g.)	*(g.)*	
I	Corn oil filled milk (prepared weekly)	10	41	829.8	1,176.3	± 22.8
II	Corn oil filled milk (prepared daily)	4	16	839.1	477.2	± 15.1
III	Hydrogenated corn oil filled milk	3	12	1,161.2	698.3	± 23.9
IV	Lard filled milk	4	10	1,256.6	557.6	± 20.6
V	Butter oil filled milk	3	12	896.8	469.2	± 20.6
VI	Fortified skimmilk	2	6	2,031.8	1,772.8	±319.7
VII	Fortified skimmilk plus corn oil	5	22	1,689.1	1,398.1	± 69.0
VIII	Fortified skimmilk plus hydrogenated soybean and cottonseed oil blend	2	8	2,063.5	1,428.4	±162.3
IX	Skimmilk	2	11	667.0	907.8	± 55.9

[a] Wet basis.

indication of the amount of diarrhea present among the various groups. The fecal output of subjects in groups with feces of apparently normal consistency (Groups II–V, inclusive) was somewhat greater than that reported by Blaxter and Wood (4) in their study of diarrhea in calves. Animals fed corn oil filled milk prepared weekly were afflicted with a severe diarrhea. In some trials, as much as 3,000 g. of feces were collected daily from individuals in this group. Tocopherol supplementation of the corn oil filled milk diets did not reduce the severity of the diarrhea. Calves receiving a corn oil filled milk prepared daily in an attempt to reduce its toxicity voided a considerably smaller amount of feces. Hydrogenation of the corn oil greatly reduced the amount of feces excreted daily. Similarly to Group I, the feces of animals on the fortified skim-milk plus corn oil diet were extremely loose.

Although the mean daily excretion of feces was greatest for the fortified skimmilk group, there was a large amount of variation in the values for that group, as shown by the standard error of the mean. Considerable variation in the consistency of the feces was also noted in the trials of animals in Group IX. The marked fecal excretion by calves fed the various fortified skimmilk diets undoubtedly was influenced to some extent by the abnormally high solids content of these rations. The factors responsible for the relatively large fecal output by the skimmilk group are not apparent. It is interesting to note, however, that Iowa workers (13) recently reported that diets high in concentrations of milk solids-not-fat and/or low in fat are likely to cause diarrhea.

Differences in the color of feces were also quite pronounced among the various experimental rations. The feces of animals on the weekly prepared corn oil filled milk diet were characteristically greenish-yellow in color; whereas, those excreted by calves receiving the same diet prepared daily were a darker green. The excretions of the lard filled milk group were usually grayish-blue or sometimes clay-like. Subjects on the whole milk, skimmilk, fortified skimmilk, and butter oil filled milk rations voided feces which were the usual brown in color. In contrast, the fecal excretions of individuals on the hydrogenated vegetable oil filled milks were invariably gray. Differences in bile secretion, intestinal fat excretion, intestinal flora, and the oxidation-reduction potential of the digestive tract, as well as other factors, may influence color of feces.

Digestibility data. The apparent dry matter digestion coefficients for several of the various diets may be found in Table 2. These data were treated statistically

TABLE 2
Apparent dry matter digestion coefficients of the various experimental diets

Diet	No. calves	No. trials	Av. dry matter digestion coefficient
Corn oil filled milk prepared weekly	8	41	81.1
Corn oil filled milk prepared daily	4	16	90.4
Hydrogenated corn oil filled milk	3	12	89.5
Lard filled milk	4	10	90.9
Butter oil filled milk	3	12	93.6

according to methods outlined by Snedecor (11) and Duncan (9, 10), to test the differences among the various treatment means at the 5% level of significance. The average dry matter digestion coefficient for the weekly prepared corn oil filled milk group was found to be significantly less than that for any one of the other diets. No other significant differences in apparent dry matter digestibility were noted among the various diets. The dry matter digestion coefficients for calves in the various fortified skimmilk groups were not included in the analyses because of the limited amount of data available for these diets.

The significantly greater apparent dry matter digestibility of the daily prepared corn oil filled milk ration is of considerable interest. Undoubtedly, a large portion of the increase in apparent dry matter digestibility may be associated with the decrease in fecal excretion and degree of diarrhea. As previously noted, the feces of calves in the daily prepared corn oil filled milk group were nearly normal in consistency. Blaxter and Wood (4) reported that the apparent digestibility of fat, protein, and ash drops from as high as 97 to as low as 40% in some instances, as the result of diarrhea in calves.

The apparent fat digestibility data are presented in Table 3. The adjusted fat digestion coefficients listed in this table are values adjusted for metabolic fecal fat as follows: The mean amount of fat excreted daily by calves in the skimmilk group was expressed as a percentage of the fecal dry matter. Since the diet of these animals contained only 0.1% fat, it was assumed that this value, 5.36%, was indicative of the metabolic fecal fat expressed as a percentage of the fecal dry matter. Thus, an amount equivalent to 5.36% of the fecal dry matter was deducted from the total fat excretion in calculating the adjusted fat digestion coefficient for calves receiving the several filled milks.

The data used in determining the metabolic fecal fat excretion were derived from 11 digestion trials conducted with two subjects in the skimmilk group. The finding that the metabolic fecal fat excretion by calves in this group amounted to 5.36% of the fecal dry matter is in very close agreement with the 5.30% value obtained under similar conditions by previous workers (5). More recently, Cunningham and Loosli (7) reported values of 3.1–5.8% when the metabolic fecal fat excreted by calves on a fat-free purified diet was expressed as a percentage of the fecal dry matter.

An analysis of variance of the unadjusted fat digestibility data indicated that significant differences existed among the various diets. Further statistical treatment revealed that the digestibility of butter oil was superior to that of the fats in each of the other diets studied. In every case this superiority of butter oil was significant at the 1% level. No other significant differences in unadjusted fat digestion coefficients existed among the remaining diets.

Significant differences were found to exist also among the adjusted fat digestion coefficients for the various rations. The digestibility of butter oil was again demonstrated to be significantly greater (1% confidence level) than that of the fats in the other diets. No other significant differences were noted among the remaining groups. It should be pointed out that both the highest and lowest fat digestion coefficients for the over-all experiment occurred in the weekly

TABLE 3

Summary of apparent fat digestibility data for calves on the various experimental diets

Diet	No. calves	No. trials	Av. unadjusted fat digestion coefficient	Av. adjusted [a] fat digestion coefficient	Free fatty acids	Soaps	Neutral fat
					(av. % fecal fat)		
Corn oil filled milk prepared weekly	8	32	84.3	88.3 ± 0.30[b]	22.9 ± 0.53[b]	42.8 ± 0.85[b]	34.3 ± 0.48[b]
Corn oil filled milk prepared daily	4	14	88.5	90.2 ± 0.36	12.7 ± 0.87	57.7 ± 1.20	29.6 ± 0.73
Hydrogenated corn oil filled milk	3	10	88.2	90.3 ± 0.54	25.7 ± 2.26	53.7 ± 2.22	20.6 ± 1.23
Lard filled milk	4	7	87.1	88.9 ± 1.06	20.2 ± 2.85	52.6 ± 2.99	27.2 ± 0.79
Butter oil filled milk	3	9	95.1	96.8 ± 0.26	25.3 ± 2.62	34.3 ± 1.70	40.4 ± 1.69
Skimmilk	2	8	—	—	24.2 ± 3.23	26.6 ± 2.92	49.2 ± 2.57

[a] Values adjusted for metabolic fecal fat as noted in text.

[b] Mean ± standard error of the mean.

prepared corn oil filled milk group. No consistent effect of collection period upon fat or dry matter digestibility was observed in any of the groups.

It is interesting to note that the apparent digestibility of the hydrogenated corn oil did not differ significantly from that of corn oil or lard. The melting point range of the hydrogenated corn oil used in this investigation was 45–48° C. Studies reported by Crockett and Deuel (6), as well as by others, have indicated that the critical melting point range above which there appears to be a marked decrease in fat digestibility is 50–55° C. The apparent digestion coefficients for hydrogenated corn oil obtained in this experiment compare favorably with those obtained by Augur, Rollman, and Deuel (2), when rats were fed hydrogenated cottonseed oil with a similar melting point. They are, however, somewhat greater than those for hydrogenated soybean oil as reported for calves by de Man (8).

The digestibility of lard in this study is somewhat less than that reported by Crockett and Deuel (6) for the rat, but somewhat greater than the 82.8% figure obtained by Cunningham and Loosli (7), when calves were fed a purified diet. The adjusted digestion coefficient of 96.8 for butterfat is somewhat greater than the 93.0 figure for calves obtained similarly by earlier workers (5). However, both the adjusted and unadjusted digestion coefficients for butterfat are in good agreement with values reported by de Man (8) as well as by Blaxter and Wood (3). It should be pointed out that the daily preparation of the corn oil filled milk did not significantly increase the digestibility of this highly unsaturated vegetable oil, although dry matter digestibility was significantly improved, as noted earlier.

The partition of fecal fats is also presented in Table 3. The finding that a considerable portion of the fecal fat is present as soaps is in agreement with previous results obtained with rats (2, 6) and calves (3). Thus, it appears important that fat determinations be made on acidified samples of fecal dry matter in fat digestibility studies, in order to include fecal soaps in the total fat excretion. It should be noted that a larger portion of fecal fat was excreted as soaps than as neutral fats and free fatty acids combined, by animals on the hydrogenated corn oil diet. Augur, Rollman, and Deuel (2) also showed this to be true in digestibility studies of hydrogenated cottonseed oil with rats. This same pattern of fecal fat excretion occurred in the daily prepared corn oil and lard filled milk groups, and was closely approximated by animals on the weekly prepared corn oil filled milk ration. Blaxter and Wood (4) obtained similar results with calves fed margarine.

A marked decrease in the percentage of fecal fat excreted as free fatty acids occurred when the corn oil filled milk diet was prepared daily. Undoubtedly, a portion of this decrease is associated with the abatement of the severe diarrhea in this group. Blaxter and Wood (4) demonstrated that a large increase in fatty acid excretion results in calves afflicted with diarrhea. Regarding the partition of the metabolic fecal fat as listed under the skimmilk diet in Table 3, the percentage of fecal fat excreted as free fatty acids closely agrees with the value obtained by Cannon, Espe, and Waide (5). The percentage of metabolic fecal fat present as neutral fat, however, is approximately 10% greater than in

the results reported by these workers. On the other hand, the portion of metabolic fecal fat present as neutral fat in this investigation is in agreement with the results obtained by Cunningham and Loosli (7). It is interesting to note the similarity between the fecal fat partition on the butter oil filled milk diet and that of what has been assumed to be metabolic fecal fat, as excreted by subjects in the skimmilk group.

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SOME EFFECTS OF FEEDING VARIOUS FILLED MILKS TO DAIRY CALVES. III. BLOOD PLASMA TOCOPHEROL AND VITAMIN A LEVELS, DIET STORAGE EFFECTS, AND EVIDENCE OF TOXICITY[1]

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SUMMARY

Low blood plasma tocopherol levels were found in calves fed corn oil filled milk, despite a relatively high tocopherol intake. Both plasma tocopherol and vitamin A values declined progressively during the experimental period in the corn oil filled milk group. Low blood plasma tocopherol levels also occurred in animals fed lard filled milk and a ration containing butter oil, which was prepared from oxidized butter. Oral supplementation of the corn oil and lard filled milk diets with 500 mg. of DL-alpha tocopherol or alpha tocopheryl acetate daily resulted in high plasma tocopherol values.

A loss of 30-40% of total tocopherol content of the corn oil filled milk resulted during a maximum storage period of seven days. It was also demonstrated that an extremely high peroxide content developed in the corn oil filled milk during a similar storage period. The feeding of a corn oil filled milk prepared daily rather than at weekly intervals markedly reduced the severity of the diarrhea and increased dry matter digestibility. Weight gains, however, were not materially affected.

The weekly prepared corn oil filled milk was found to be as detrimental for calves 4 mo. old as for younger animals. The possibility that corn oil filled milk is toxic to dairy calves is discussed in considerable detail.

Since the presence of highly unsaturated fats and rancid fats, in general, has been associated by various workers (18, 19) with the destruction of fat-soluble vitamins or alterations in the body requirements for such factors, a study was undertaken of blood plasma tocopherol and vitamin A levels in calves fed various filled milks. The symptoms of muscular involvement reported previously (1) as occurring in animals fed corn oil filled milk further suggested the necessity of such a study.

Other phases of the investigation presented here were conducted because of evidence reported by other workers (7, 21) that highly oxidized fats also exert a direct toxic effect upon the body.

EXPERIMENTAL PROCEDURE

The methods used in the feeding and management of the subjects have been published earlier (1, 2). Approximately 50-ml. samples of jugular blood were

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drawn at intervals of from ten to 20 days throughout the experimental period. All pre-experimental samples were obtained when the calves were from ten to 14 days of age. The plasma was separated from the cellular components and stored at −4° C. until assayed for tocopherol and, in some cases, vitamin A content. The storage period never exceeded 2 wk.

For the determination of blood plasma tocopherol levels, aliquots containing 5.0 ml. of blood plasma were extracted with petroleum ether (b.p. 63–67° C.), as outlined by Kimble (16) and Quaife and Harris (24). The total fat-soluble reducing substances were determined colorimetrically, using the Emmerie and Engel reaction (11), in accordance with procedures employed by Devlin and Matill (9). Based on research reported by Kaunitz and Beaver (15), a correction was made for color inhibition by plasma extracts. Since most calves had low blood plasma carotene values, the correction for carotene interference was small, or negligible. All values were expressed as micrograms of alpha tocopherol per 100 ml. of blood plasma. In addition to the blood plasma tocopherol estimates, determinations of total fat-soluble reducing materials were also made on aliquots of the corn oil filled milk, employing generally the same procedures used in blood plasma analyses.

Blood plasma vitamin A determinations were made on 10-ml. samples obtained as outlined above. The method used was essentially that described by Kimble (16), but with mild saponification as given by Bessey *et al.* (5). The vitamin A was measured colorimetrically by means of the Carr-Price reaction. The total Carr-Price reacting materials were calculated and expressed as micrograms of vitamin A per 100 ml. of blood plasma.

Several methods of tocopherol administration and types of tocopherol supplements were investigated. In every instance, an equivalent of 500 mg. of DL-alpha tocopherol or alpha tocopheryl acetate was administered daily to each calf. All orally administered tocopherol supplements were fed in gelatin capsules immediately prior to the P.M. feeding. Sesame oil served as a carrier for both the oral and intramuscular administration of alpha tocopheryl acetate. No carrier was used with the orally administered DL-alpha tocopherol. Whereas the orally supplemented calves were given 500 mg. of tocopherol supplement daily, the subjects in the intramuscularly administered group received 1,500 mg. of alpha tocopheryl acetate every three days.

Peroxide values were obtained on samples of the filled milks by a modification of the Hills and Thiel method (12), as developed by Stine *et al.* (26). This method is based on the oxidation of the ferrous ion to ferric in the presence of peroxides, and the resulting formation of ferric thiocyanate.

RESULTS AND DISCUSSION

Tocopherol determinations. The mean plasma tocopherol levels of the various groups for the experimental period of 76 days, and the "t" values for certain comparisons, are given in Table 1. The relatively low mean tocopherol estimate for the corn oil filled milk group is of considerable interest, in view of the tocopherol content of corn oil (29). The mean for the corn oil filled milk group

TABLE 1

Mean total fat-soluble reducing substances, expressed as micrograms of alpha tocopherol per 100 ml. blood plasma

Diet	No. calves	No. determinations	Mean tocopherol value micrograms %	t-value
I. Comparison of corn oil filled milk with various other diets				
Corn oil filled milk	9	34	137	.....
Hydrogenated corn oil filled milk	4	19	187	2.09*
Hydrogenated cottonseed and soybean oil blend filled milk	3	6	468	3.21**
Lard filled milk	4	20	93	3.34**
Butter oil filled milk	3	15	90	4.35**
Fortified skimmilk	3	14	45	7.76**
Whole milk	2	14	314	5.97**
II. Comparison of the oral tocopherol supplemented corn oil filled milk ration with various other diets				
Corn oil filled milk with oral DL-alpha tocopherol supplementation [a]	3	14	774	.....
Corn oil filled milk with oral alpha tocopheryl acetate supplementation [a]	4	15	708	0.78
Corn oil filled milk with intramuscular alpha tocopheryl acetate supplementation [a]	3	15	379	5.48**
III. Comparison of lard filled milk ration with the oral tocopherol supplemented lard filled milk diet				
Lard filled milk	4	20	93	.....
Lard filled milk with oral alpha tocopheryl acetate supplementation [a]	2	10	584	9.44**

* Significant at the 5% level.

** Significant at the 1% level.

[a] All animals received an equivalent of 500 mg. of alpha tocopherol daily. See Procedure section for further details.

given in Table 1 does not include determinations obtained from the last three subjects raised on this diet. A mean estimate of 200 μg. of alpha tocopherol per 100 ml. of blood plasma resulted in these particular calves. It is felt that this discrepancy in results might stem from a change in the time of blood collection. Whereas all other blood samples were collected approximately 3 hr. after the morning feeding, these were obtained approximately 6 hr. following the morning allowance of the ration. Barker and Jacobson (3) have demonstrated that the plasma "fat" values of calves receiving a highly unsaturated vegetable oil increase to a maximum at the end of 6 hr. after feeding. Because of the tocopherol content of corn oil and this plasma fat pattern, it is quite conceivable that the time of collection may have influenced the plasma tocopherol levels of these subjects.

The considerable difference noted between the mean plasma tocopherol values obtained for the hydrogenated corn oil and hydrogenated cottonseed and soybean oil blend calves can not be explained on the basis of available data. It should be noted, however, that the levels found in the latter group are of the order expected, in view of the tocopherol content of vegetable oils. The hydrogenated corn oil used in this study was prepared from refined corn oil, specifically for the purpose of this investigation. The tocopherol content of the corn oil may have been deleteriously affected by the processes used in the hydrogenation of the product in the pilot-sized operation. No data, however, are available on the tocopherol content either of the hydrogenated corn oil or of the hydrogenated cottonseed and soybean oil blend. The difference in mean plasma tocopherol values between the whole milk and butter oil filled milk groups also requires elaboration. Undoubtedly, the low plasma tocopherol levels found in butter oil filled milk calves may have resulted from a possible low tocopherol content of the butter oil. This product was prepared from oxidized butter and gave higher than usual peroxide values when incorporated in the filled milk. The low blood plasma tocopherol values found in the lard filled milk group presumably were largely a reflection of the notoriously low tocopherol content of lard. Similarly, the extremely low levels obtained from the calves raised on the fortified skimmilk ration were clearly a reflection of the low tocopherol content of the diet.

When administered orally, DL-alpha tocopherol and alpha tocopheryl acetate were equally effective in maintaining high levels of tocopherol in the blood plasma of animals fed corn oil filled milk. However, intramuscular administration of alpha tocopheryl acetate failed to raise the plasma tocopherol values to levels as high as did oral supplementation. British workers (6) reported that no increase in blood plasma concentrations of tocopherol occurred when alpha tocopheryl acetate was administered intramuscularly. Other investigators (25) found that subcutaneous injections of alpha tocopheryl acetate gave only slight or no increase in blood plasma tocopherol values. These discrepancies in the results of parenteral administration may be due, in part, to differences in dosages and sampling times, as well as to other factors.

The tocopherol values for all groups declined during the first 20 days of experimental feeding. This period coincided with the 15-35 days of age interval

for the subjects. Parrish *et al.* (22) have demonstrated that such a decrease in fat-soluble vitamins occurs in the blood plasma of normal calves during the 1st mo. of life. A statistically significant difference in blood plasma tocopherol levels due to sampling periods beyond 35 days of age was found only in the corn oil filled milk group. A mean of 180 μg. of tocopherol per 100 ml. of blood plasma following 20 days of corn oil feeding diminished to a mean of 115 μg. at the end of 70 days. This steady decline in plasma tocopherol values occurred despite the ingestion of increasingly greater amounts of a vegetable oil with a relatively high tocopherol content. A similar decrease in plasma tocopherol concentrations was encountered by Viswanatha, Gander, and Liener (28) when rats were fed a corn oil–lactose diet.

To ascertain the effects of storage on the tocopherol content of the weekly prepared corn oil filled milk, serial determinations of the total fat-soluble reducing substances were made on the ration stored at 5° C. As shown in Table 2, the results indicate that a 30–40% decrease in total tocopherol content occurred during a maximum storage period of seven days. The total tocopherol content of the corn oil immediately following the preparation of the diet closely resembled that reported by Willy (29). It has been shown previously, however, that the alpha tocopherol content of corn oil is only 12.6 mg. per 100 g. of oil. The major portion of the total tocopherols in corn oil is of the gamma form. Embree (10), among others, has demonstrated that alpha tocopherol has a higher biological activity than other forms.

TABLE 2

Effect of storage on the tocopherol content of corn oil filled milk

Days stored	Total tocopherol[a]		Tocopherol loss	
	(mg/100 ml. corn oil)		*(%)*	
	Sample 1	Sample 2	Sample 1	Sample 2
0	91	112	0	0
1	79	104	12	8
2	74	88	18	21
3	74	89	18	20
4	71	69	21	38
5	68	....	24	....
7	62	65	31	42

[a] Total fat-soluble reducing materials calculated as gamma tocopherol from 20-min. readings.

Although a 30–40% loss in the total tocopherol content of corn oil may have occurred during the maximum storage period, the alpha tocopherol content of the corn oil fed the subjects should have exceeded that of butterfat, unless it was preferentially destroyed. Lundborg (17) found that butterfat contained 3.0 mg. % of tocopherol, or less, all of which is presumed to be of the alpha form. Thus, the relatively low mean blood plasma tocopherol values obtained in the corn oil filled milk group occurred despite an apparently adequate intake of alpha tocopherol. The decline of plasma tocopherol values may have resulted from a continual increase in tissue requirements for vitamin E, as influenced in some manner by the highly unsaturated fat in the ration. Mason and Filer (18) have suggested that lard and cod liver oil in the diet influence the develop-

ment of vitamin E deficiency symptoms by causing autoxidation of the vitamin during storage, or digestion of the diet and/or increasing the tissue requirements for tocopherol in some unknown manner. However, the possibility that a portion of the decline may have resulted from the prolonged diarrhea must not be overlooked, in view of the report by Thomas and Okamoto (27).

Vitamin A values. As with the tocopherol determinations, good agreement was obtained between duplicate analyses, as well as among values for plasma samples frozen and assayed at regular intervals. For reasons detailed later, plasma vitamin A determinations were conducted only on calves in the corn oil or hydrogenated cottonseed oil filled milk and whole milk groups. Statistical treatment of the vitamin A data revealed that a significant difference due to sampling periods occurred only on the corn oil filled milk diet. This decline in blood plasma vitamin A values is shown in Figure 1. The 15-day values represent the pre-experimental determinations. Similarly to the tocopherol levels, the vitamin A estimates for all groups decreased during the 15–35 days of age period. As noted earlier, results presented by previous workers (22) have indicated that such a decline occurs in normal calves during this period. The decrease in plasma vitamin A concentrations in the corn oil filled milk group follows the same pattern as did the tocopherol values and may be the result of similar factors already mentioned. Statistical treatment of the data indicated that the mean plasma vitamin A level for the corn oil filled milk calves was significantly lower than that of the hydrogenated cottonseed oil or whole milk group at the 5 and 1% confidence levels, respectively.

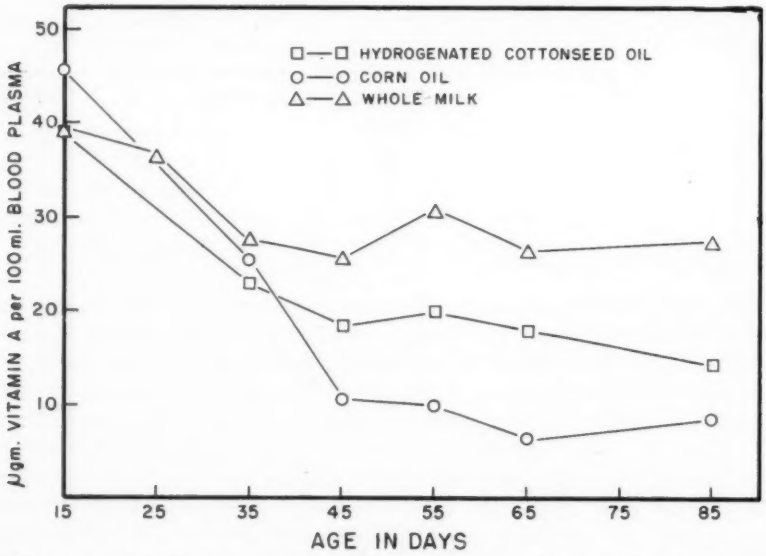

FIG. 1. Mean blood plasma vitamin A estimates of calves fed corn oil or hydrogenated cottonseed oil filled milks and whole milk.

The lowest blood plasma vitamin A values obtained from calves fed corn oil filled milk merely approached the borderline levels demonstrated by Boyer *et al.* (7) to be necessary for adequate vitamin A nutrition. Since a vitamin A deficiency in the corn oil filled milk animals did not appear to be a primary factor on the basis of the blood plasma levels or histopathological findings, further vitamin A determinations were not conducted on subsequent groups. The effects of the corn oil filled milk diet on blood plasma vitamin A concentrations as reported here are similar to those observed by Murley *et al.* (21) in animals receiving soybean oil filled milk. These workers also concluded that a vitamin A deficiency was not a primary factor responsible for the unsatisfactory results obtained when calves were fed a filled milk containing a highly unsaturated vegetable oil. In the present experiment, calves on the filled milks received 160 I.U. of supplemental vitamin A per pound of body weight daily. Whole milk calves received no supplemental vitamin A.

Peroxide content of the several rations. In light of the adverse effect of storage on the tocopherol content of corn oil filled milk, it was deemed advisable to obtain a measure of the peroxide content of the various filled milks. The results of this study are shown in Figure 2. A rapid rise in peroxide values, expressed as milliequivalents of O_2 per kilogram of fat, was noted in the corn oil filled milk during a maximum storage period of seven days at 5° C. The continual increase in the peroxide levels of the corn oil filled milk is in sharp contrast to the relatively constant values obtained with the other filled milks. It should be noted that the peroxide concentrations in the butter oil filled milk were considerably higher than those generally found in butterfat.

Evidence of toxicity. The development of extremely high peroxide values in the corn oil filled milk during storage prompted a study with a corn oil filled

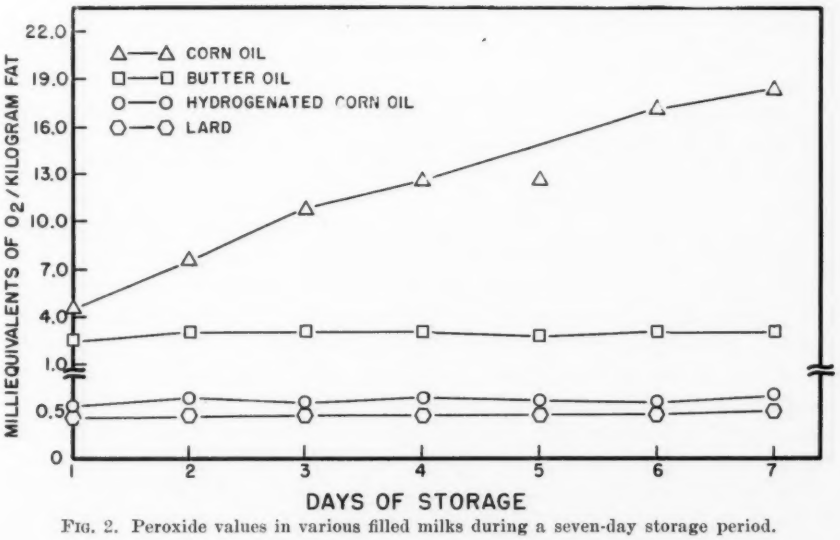

FIG. 2. Peroxide values in various filled milks during a seven-day storage period.

milk prepared daily rather than at weekly intervals. A group of six calves were fed this diet, having a peroxide content which should not have exceeded 5 meq. of O_2 per kilogram of fat. As mentioned in an earlier report (2), the severe diarrhea present in the animals disappeared when the ration was prepared daily. The mean daily fecal excretion of the subjects approached that for individuals receiving butter oil, lard, or hydrogenated corn oil filled milks (2). In addition, the apparent dry matter digestibility of the daily prepared ration was significantly greater than that of the same diet prepared weekly. The apparent fat digestion coefficients for the daily prepared group were also somewhat higher than those for the calves fed the weekly prepared corn oil filled milk. These differences in fat digestibility, however, were not statistically significant (2).

Despite the beneficial effects of daily preparation noted here, no appreciable improvement was noted in the average daily gains made by these animals, as compared to those of subjects receiving the corn oil filled milk prepared at weekly intervals. The average daily gain at the end of 42 days of experimental feeding for the daily prepared group was 0.92 lb., as compared to 0.86 lb. for the calves on the regular corn oil filled milk regime. Since the animals fed the daily prepared corn oil filled milk were on the experiment for only 42 days, owing to a lack of time and funds, insufficient tocopherol determinations were made to ascertain the effects of this diet on blood plasma tocopherol values. No necropsy studies were performed on these calves, for the same reason. The results obtained by feeding a daily prepared corn oil filled milk indicate that peroxide levels greater than 5.0 meq. of O_2 per kilogram of fat, as measured by the method of Stine *et al.* (26), or factors associated with peroxide levels of that magnitude, were undoubtedly responsible for the pronounced diarrhea and low dry matter digestibility observed in calves fed the weekly prepared ration. The data suggest, however, that the severe diarrhea and low dry matter digestibility per se were not responsible for the poor gains made by the corn oil filled milk calves

Other results obtained in this investigation also suggest that the feeding of a corn oil filled milk to calves depresses growth in some manner (1). Average gains made by animals fed corn oil filled milk were slightly less than those of subjects fed a calorically subnormal diet of skimmilk. Similarly, average gains for calves receiving the fortified skimmilk ration containing 3.5% corn oil were somewhat lower than those of individuals fed the fortified skimmilk without additional fat. This situation is in sharp contrast to the markedly greater gains noted when 3.5% hydrogenated vegetable oil was included in the fortified skimmilk ration. The growth-depressing effect of corn oil appears to transcend the differences in digestibility presented in an earlier report (2).

Additional studies were made to obtain further evidence of a possible toxic effect resulting from corn oil feeding. One calf near death from corn oil feeding was placed on a ration consisting of equal parts of corn oil filled milk and whole milk. The feces of this calf appeared normal within five days and all signs of weakness disappeared by the end of a nine-day period. The animal

was sold on the market 32 days following the change in diet. Before the change, the average daily gain of the subject was 0.43 lb., as compared to 1.46 lb. on the ration containing equal parts of corn oil filled milk and whole milk.

In Figure 3 may be found the growth curves of two additional animals which were used to note the effects of the corn oil filled milk on the well-being of older dairy calves, and to obtain further information on the effects of replacing portions of the corn oil filled milk with other rations. Both subjects were Holstein bull calves weighing approximately 275 and 285 lb., respectively, when removed from a ration of skimmilk, alfalfa hay, and a concentrate mixture at 115 days of age. During a ten-day transitional period, these individuals received

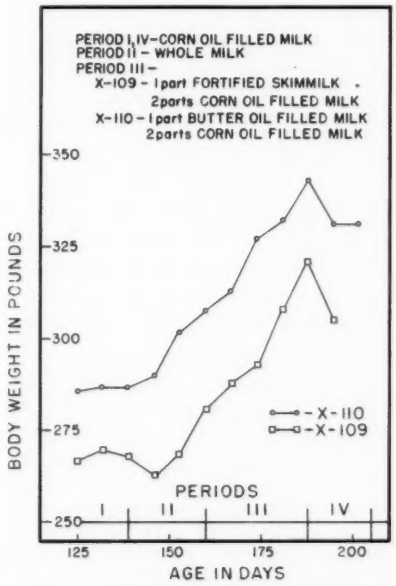

FIG. 3. Growth curves of two calves placed on the corn oil filled milk diet at 115 days of age.

only whole milk. By the end of this period, the weight loss due to the removal of fill and the somewhat drastic change in the diet had ceased. The calves were then placed on the corn oil filled milk ration. Following slightly more than 2 wk. of experimental feeding, the animals were exhibiting most of the symptoms already mentioned as occurring in corn oil filled milk subjects (1). The improved weight gains noted when only one-third of the daily allowance of the ration consisted of a suitable diet are shown in Figure 3. This improvement occurred in spite of the persistence of the diarrhea and low blood plasma tocopherol levels in both calves. At 190 days of age, both animals were again placed on the corn oil filled milk diet. A sharp decline in weight resulted and symptoms of muscular involvement appeared within seven to ten days. The physical

condition of both subjects had deteriorated within this period to the point where it was necessary to sacrifice them in order to insure accurate necropsy findings.

On the basis of the limited evidence presented in this paper and reports in the literature, it is felt that a considerable portion of the improvement noted in the physical condition and gains of calves fed as little as one-third of the diet in the form of a satisfactory ration, may have been due to a reduction in the intake of a toxic factor(s) present in the corn oil filled milk. Apparently, the tolerance of calves for toxic factors, arising from the use of highly unsaturated fats under these experimental conditions, is much lower than that of young pigs fed similar diets. McConnell (20) and others have shown that the use of highly unsaturated vegetable oils in formulas for baby pigs gives results comparable to those obtained with hydrogenated vegetable oils and animal fats. Data reported by others (4) also suggest that the length of the storage period for filled milks may influence the condition of calves fed such rations. Fewer toxic effects also were reported by Iowa workers (14) when the level of highly unsaturated vegetable oil in the diet of calves was reduced from 3 to 2% of the ration.

The findings reported here suggest that growth may be depressed by feeding a corn oil filled milk to dairy calves. Such a suggestion is in general agreement with evidence cited by Burr and Barnes (8), as well as by Quackenbush (23), that oxidized or rancid fats exert a direct toxic effect which is manifested in the body tissues. It has been demonstrated by contemporary workers (13) that various substances having conjugated double-bond systems in common aggravated dermal lesions and decreased growth in fat-deficient rats. It is unfortunate that the effect on weight gains was not studied with a corn oil filled milk having a peroxide content as low as that of the butter oil or hydrogenated corn oil filled milk rations which produced satisfactory gains.

ACKNOWLEDGMENTS

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SOME EFFECTS OF FEEDING VARIOUS FILLED MILKS TO DAIRY CALVES. IV. NECROPSY FINDINGS, ELECTROCARDIOGRAPHIC STUDIES, AND CREATINURIA RATIOS[1]

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SUMMARY

Gross and microscopic lesions were demonstrated in the cardiac and skeletal muscles of calves fed corn oil or lard filled milk. Such lesions were also found in animals receiving a similar ration containing butter oil, which had been prepared from oxidized butter. Both oral and intramuscular supplementation of the corn oil and lard filled milk diets with high levels of tocopherol prevented the development of muscular involvement during the course of the experiment. No evidence of muscular involvement was encountered in calves fed a low-fat, tocopherol-poor ration for periods as long as 5.6 mo. The influence of certain fats in the diet on the production of so-called vitamin E deficiency lesions in cattle is discussed.

The microscopic lesions in cardiac and skeletal muscles are characterized in detail. In general, these lesions were typical of those described previously as occurring in white muscle disease or nutritional muscular dystrophy. Necrospy findings, other than muscular lesions, are also presented.

Marked changes occurred in the electrocardiographic pattern of subjects in the corn oil filled milk group. The factor(s) responsible for these gross alterations could not be ascertained from the available data. The determination of creatinuria ratios did not provide a means of following the development of muscular involvement in calves.

The symptoms of muscular involvement noted in corn oil filled milk calves (3) suggested the need for a detailed necropsy examination of the animals fed the various experimental diets. The electrocardiographic studies and creatinuria ratios reported here represent an attempt to follow the development of the muscular lesions, which were demonstrated in calves receiving certain filled milks.

Details regarding the feeding and management of the experimental subjects in this report have been presented previously (1, 2, 3).

EXPERIMENTAL PROCEDURE

Complete necropsy studies were performed on the experimental calves which were sacrificed at the end of 90 days of age or had died before completing the experimental period. The extremely poor condition of some animals required that these subjects be euthanized by electrocution to avoid post-mortem changes. It should be added that some individuals from several groups were not sacrificed

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until 5–6 mo. of age. All endocrine glands and other organs were weighed following the usual gross observations. Sections for microscopic examinations were taken from the various glands and organs, as well as from cardiac and skeletal muscles. Skeletal muscle sections were obtained from the rhomboideus and triceps groups of the thoracic limb; while others were taken from the semimembranosus, semitendinosus, and gastrocnemius of the pelvic limb. Heart sections were obtained throughout the walls of both ventricles, taking care to include sections of the Purkinje network. All sections were placed in a 10% formalin fixative. Except as noted otherwise, an H & E strain was employed, using Harris hematoxylin and acid alcohol eosin.

Electrocardiographic tracings were obtained from each calf at regular intervals, using a Sanborn Viso-cardiette. A pre-experimental record was made at from ten to 14 days of age. Final electrocardiograms were obtained at 90 days of age, or prior to death in animals unable to complete the experimental period. All tracings were taken with the calf in its usual pen. A standard recording speed of 25 mm. was employed. Since research conducted by Sellers *et al.* (19) with cattle indicated that Lead IV (precordial) appears to give the most standard and duplicable results from animal to animal, only this lead was recorded. The precordial lead is obtained by placing bipolar electrodes over the apex of the heart and the top of the right scapula. All measurements of the electrocardiograms were made as described by Burch and Winsor (10). Because recent studies by Sellers *et al.* (19) have shown that a single rather than bi- or triphasic pattern of the QRS complex often occurs in the precordial lead (Lead IV) of records obtained from cattle, the amplitude of the QRS complex was measured, rather than the potentials of the individual Q, R, and S waves.

Urine was collected from the calves for the periods during which the animals were in the digestion stalls (1). The total urine output was measured daily and composite samples taken for analysis. Creatine and creatinine values were obtained, using the procedure outlined by Folin (12). Creatinuria ratios were calculated by the method of Eppstein and Morgulis (11), as used by Hove and Harris (15).

RESULTS AND DISCUSSION

Necropsy findings. Except as noted otherwise, no appreciable differences in gland or organ weights were observed which might be attributed to the levels of fat in the diet, type of fat in the ration, solids content of the diet, or vitamin E status of the calves. All figures were on a wet-weight basis and were expressed as grams per kilogram of body weight. The variation among calves as well as diets appeared to be surprisingly small for most glands and organs. However, the values obtained for the thymus and spleen were somewhat more erratic.

It should be noted that several individual values on various diets differed markedly from the over-all or group mean. One animal out of 13 in the corn oil filled milk group had a heart value of 22.2 g. per kilogram of body weight, which is considerably above the over-all mean of 6.3. The heart values of other subjects on this diet approximated the over-all mean. Large thyroid figures

were obtained from four of the calves in the corn oil filled milk group. The thyroid value for these subjects averaged 0.9 g., as compared to the over-all mean of 0.3 g. per kilogram of body weight. All other thyroid values in this group of 13 animals closely resembled the over-all mean. An enlarged thyroid value also was found in one out of four individuals fed hydrogenated corn oil filled milk. It did not appear that gains were influenced by the presence of an enlarged thyroid.

Gross lesions, excluding those observed in muscle, were infrequently encountered. Those noted in the respiratory system were secondary pneumonia associated with various degrees of atelectasis, and consolidation. Such lesions were demonstrated grossly in six of 16 subjects in the corn oil filled milk group. Single instances were found in groups receiving corn oil filled milk with oral tocopherol supplementation, lard filled milk, and fortified skimmilk with corn oil. These findings substantiate the earlier observation (3) that calves fed corn oil were more susceptible to infection. Gross examination of the genito-urinary system revealed white foci on the kidney of two corn oil filled milk animals. The appearance of the kidney was typical of that described as white-spotted kidney and was not thought to be associated with a vitamin E deficiency. Very little, if any, depot fat was found in subjects receiving the various corn oil filled milk diets. The absence of noteworthy lesions in the intestinal tract is of considerable interest, in view of the marked diarrhea which persisted in these animals.

The microscopic lesions, other than those in cardiac and skeletal muscles, were not of great significance. Numerous instances of mild fatty metamorphosis of the liver were observed in animals on the several corn oil filled milk rations. One such case also occurred in the lard filled milk group. Microscopic examination of the various glands and other organs revealed no lesions which would suggest trends of dietary origin. The enlarged thyroids found in some individuals did not differ histologically from the normal-sized glands. No remarkable lesions were found in the enlarged heart noted in one of the corn oil filled milk animals.

When present, the gross lesions in skeletal muscles appeared as pale or white areas. In some cases, entire muscles of both the fore and rear limbs were affected; whereas, in others only scattered areas were found, usually in the rear quarters. The lesions always occurred bilaterally and were noted in calves fed corn oil, lard, and butter oil filled milks. The gross lesions, however, were observed in approximately only 40% of the subjects with demonstrable microscopic lesions. The extensive gross lesions shown in Figure 1 were found in a calf severely afflicted with muscular involvement. A pale or white area from this animal is contrasted with normal-appearing muscle taken from the same subject.

The gross lesions observed in cardiac muscle appeared as pale or white streaks in the ventricles, or as pale patches in the septal myocardium and papillary muscles. Gross cardiac lesions were not present in all calves having histologically demonstrated lesions. The degree of muscular involvement as well as other factors may have been responsible for this failure to observe gross lesions in all subjects, in which microscopic lesions were found in cardiac and skeletal muscles.

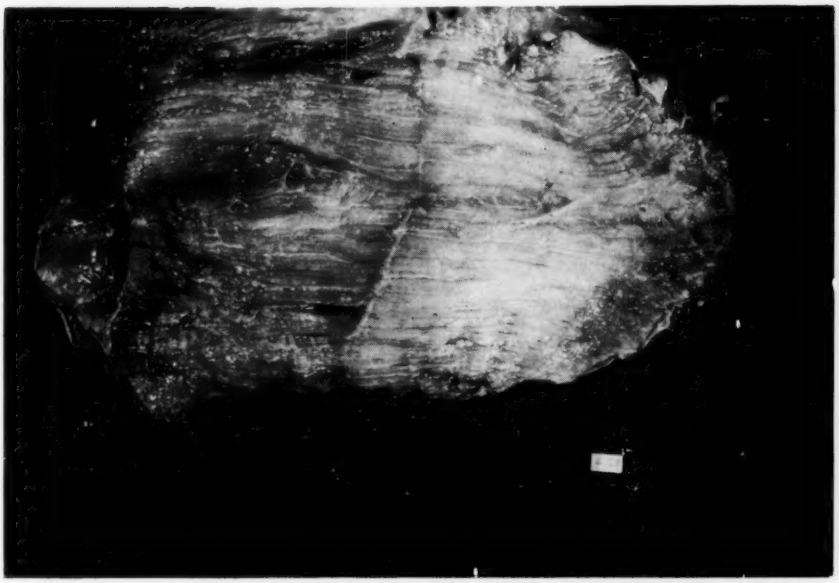


FIG. 1. Gross lesions in skeletal muscle removed from a calf afflicted with nutritional muscular dystrophy. Light area: affected muscle; dark area: normal muscle.

It should be added that the hearts removed from several individuals in the corn oil and lard filled groups were flabby.

The incidence of microscopic lesions in skeletal and cardiac muscles of calves on the various diets may be found in Table 1. Numerous animals in the corn oil and lard filled milk groups were afflicted with muscular lesions which could be demonstrated histologically. Similar lesions were also detected in two of the subjects receiving butter oil filled milk with a relatively high peroxide content (2). No microscopic lesions were observed, however, in animals fed a whole milk ration prepared and stored under similar conditions. The development of muscular lesions in calves receiving corn oil filled milk is of considerable importance. Previously, nutritional muscular dystrophy or white muscle disease has been produced experimentally only in calves fed lard or various fish oils (6, 7, 18). The presence of muscular lesions in the subjects receiving a butter oil prepared from oxidized butter is of equal significance. On each of the dystrophy-producing diets, no outward symptoms of muscular involvement were noted in several animals exhibiting microscopic lesions upon necropsy.

No muscular lesions were demonstrated in the corn oil or lard filled milk groups supplemented either orally or intramuscularly with the equivalent of 500 mg. of DL-alpha tocopherol or alpha tocopheryl acetate daily (2). Likewise, no muscular involvement was found in calves fed hydrogenated vegetable oil filled milks. Of greater importance is the finding that no muscular involvement

TABLE 1
Incidence of microscopic muscular lesions among the experimental calves

Diet	No. subjects autopsied	No. of animals with muscular lesions	
		Cardiac	Skeletal
Corn oil filled milk	16	4	12
Corn oil filled with oral tocopherol supplementation[a]	7	0	0
Corn oil filled milk with intramuscular tocopherol supplementation[a]	4	0	0
Hydrogenated corn oil filled milk	4	0	0
Hydrogenated cottonseed and soybean oil blend filled milk	2	0	0
Lard filled milk	6	3	3
Lard filled milk with oral tocopherol supplementation[a]	2	0	0
Butter oil filled milk	3	1	2
Fortified skimmilk	4	0	0
Fortified skimmilk with corn oil (including oral tocopherol supplementation)[a]	6	0	0
Fortified skimmilk with hydrogenated cottonseed and soybean oil blend	2	0	0
Skimmilk (including oral tocopherol supplementation)[a]	3	0	0
Whole milk	3	0	0

[a] See previous report (2) for details on tocopherol supplementation.

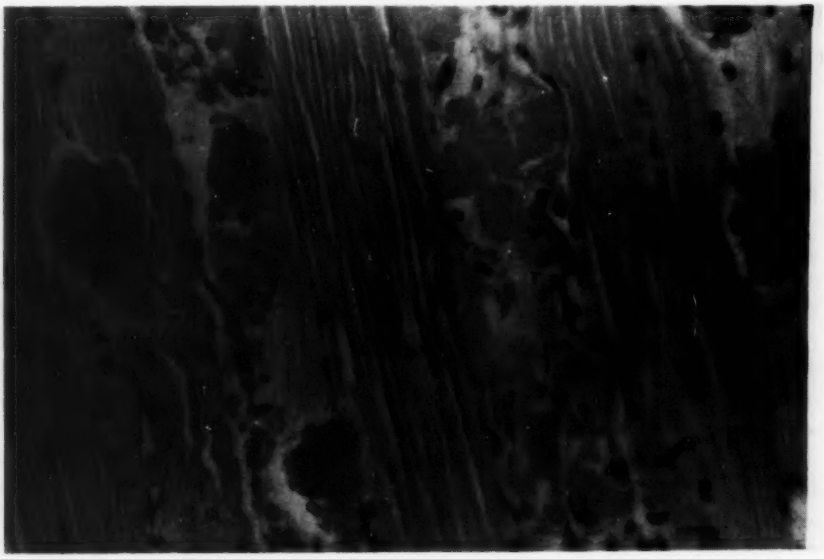


FIG. 2. Skeletal muscle fibers in various stages of degeneration. Magnification × 100.

or lesions occurred in subjects fed the low-fat and tocopherol-poor fortified skimmilk diet.

It appears that single muscle fibers and groups of fibers in certain areas are affected in the development of microscopic lesions in skeletal and cardiac muscle. Typical changes in an affected muscle may be found in Figure 2. Some fibers remain normal in appearance; whereas, adjacent fibers show signs of degeneration. As presented in Figure 3, the affected fibers appear to rupture at the Z line, forming retraction caps. Degeneration and necrosis apparently occur simultaneously with proliferation of the sarcolemmal nuclei and myocytes (Figure 2). The muscle fibers progressively show degeneration, necrosis, and fragmentation. Striations persist, however, and are easily recognized in remaining remnants. Within a short distance along a fiber the destruction varies in pattern from hyaline necrosis to atrophy. In atrophied areas the sarcolemma persists as a tube. The majority of the cells present in the later stages of degeneration appear to be sarcolemmal cells, along with a few lymphocytes. These cells are grouped around remnants of degenerating muscle fibers, forming pseudo-giant cells as shown in Figure 4. The centers of the pseudo-giant cells frequently contain

FIG. 3. Skeletal muscle rupturing at Z line. Magnification × 500.

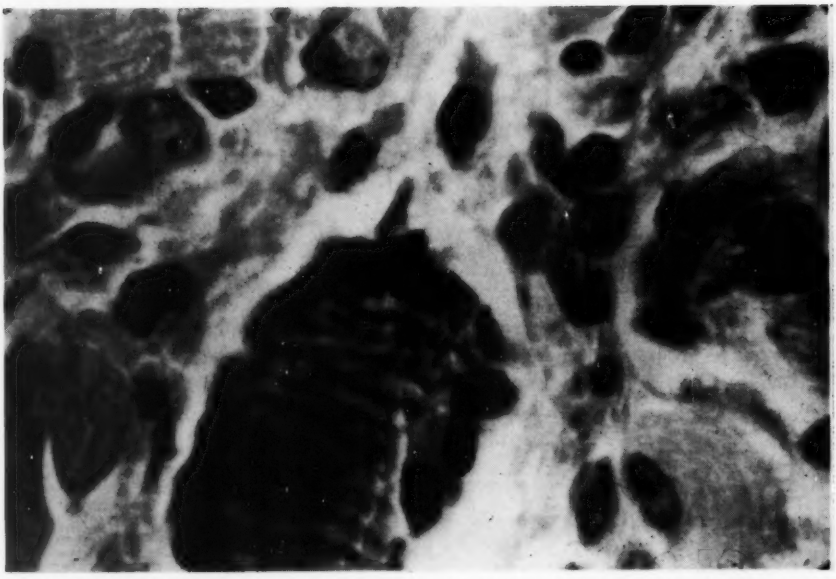


FIG. 4. Detail of a pseudo-giant cell as found in skeletal muscle lesions. Magnification × 800.

calcium, which can be demonstrated by staining with silver nitrate. Cardiac muscle changes as presented in Figure 5 were similar to those observed in skeletal muscle. However, they occurred less frequently.

The microscopic findings reported here are in general agreement with those presented by other workers. MacDonald *et al.* (16) as well as Vawter and Records (21) mention the presence of polymorphonuclear leukocytes. None was found, however, in this investigation. The lesions observed in the present study are in close agreement with those reported by Safford, Swingle, and Marsh (18).

The mean blood plasma tocopherol values were relatively low for all groups in which muscular involvement was detected (2). Lard is known to be relatively low in tocopherol, and it has been suggested that the butter oil used in this study may have had a low tocopherol content, as it was prepared from oxidized butter. However, the tocopherol content of the corn oil diet should have been adequate, as mentioned previously (2). Relatively high peroxide levels occurred in the corn oil and butter oil filled milk diets which produced nutritional muscular dystrophy (2). The peroxide content of the lard filled milk ration, however, was slightly less than that of the hydrogenated corn oil diet upon which no muscular involvement was found.

The evidence obtained in this investigation suggests that nutritional muscular dystrophy or white muscle disease, as it has been produced experimentally in calves, may not be the result of an uncomplicated vitamin E or tocopherol deficiency. In this experiment, the condition was found only in calves fed a highly unsaturated fat (corn oil), or fats with a relatively low tocopherol con-

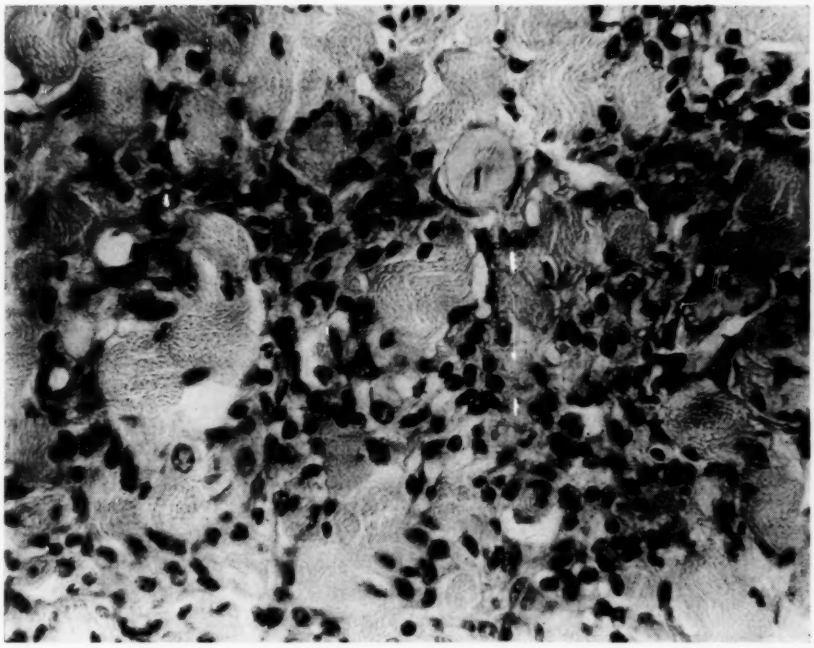


FIG. 5. Well-advanced lesion in cardiac muscle. Majority of nuclei are proliferating sarcolemma cells. Magnification × 409.

tent and an appreciable amount of unsaturated fatty acids (lard and butter oil). Despite extremely low blood plasma tocopherol concentrations, no evidence of muscular involvement was observed in animals fed a low-fat, tocopherol-poor ration (fortified skimmilk) to 5–6 mo. of age. Further support of the above suggestion is found in research conducted by contemporary workers. Blaxter *et al.* (6) concluded that the toxicity and nutritional muscular dystrophy noted in calves fed cod liver oil was due to the polyunsaturated fatty acid content of the oil and not to hypervitaminosis A or D. It should be noted that Brown (9) has demonstrated the presence of tocopherol in cod liver oil.

Singsen *et al.* (20) reported that chicks fed a basal ration containing only a small amount of tocopherol exhibited good growth, low mortality, and no encephalomalacia, in spite of low blood tocopherol concentrations. These workers stated that a low vitamin E condition per se does not result in encephalomalacia unless a stress condition, such as the presence of certain fats and oils, is placed upon the chicks. In view of present information, it is conceivable that the development of electrocardiographic changes, sudden deaths, and heart lesions noted in dairy cows fed a vitamin E–deficient ration (13, 14) may have been due to the presence of lard in the diet, rather than to a vitamin E deficiency per se. The earlier suggestion of Mason and Filer (17), that certain fats and oils in the diet influence the development of so-called vitamin E–deficiency

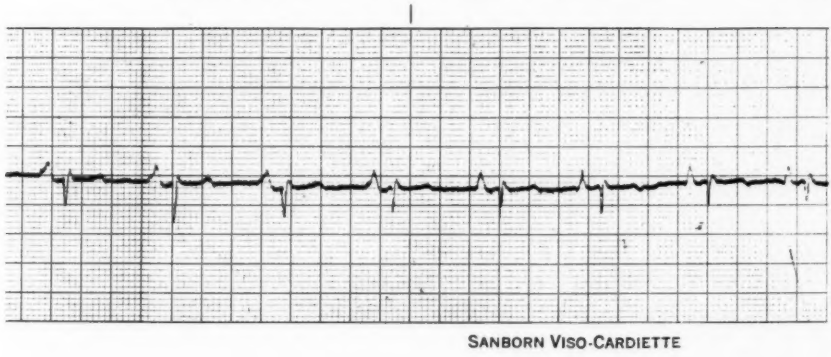
symptoms, by causing autoxidation to tocopherol during storage or digestion of the diet, and/or by increasing the tissue requirements for vitamin E in some unknown manner, remains plausible, in light of the findings reported here.

Electrocardiographic studies. Alfredson and Sykes (4) described the effects of heart rate on interval duration, as well as those variations within animals which may be attributed to serial tracings in normal cattle. More recently, Sellers *et al.* (19) obtained data concerning the individual variations in wave potentials as they occur in Lead IV (precordial) of tracings from normal dairy cattle. Thus, the changes and values obtained in this study were analyzed on an individual basis with these and other considerations in mind. As a result, significant changes which appeared to be of dietary origin were found only in the corn oil filled milk group. These changes consisted of increased potentials of the QRS complex and T wave, as well as an increased duration of the QRS interval.

Such gross alterations and others were noted in electrocardiograms obtained from three of five subjects fed the corn oil filled milk without tocopherol supplementation. Serial tracings of Calf E851 are shown in Figure 6. When compared with the pre-experimental record, the final record of this animal is characterized by increased potentials of the QRS complex and T wave, as well as by an inversion of the T wave. A marked widening of the QRS interval also occurred, and the P wave is not discernible in the tracing obtained two days before death. A reflection of the somatic tremors exhibited by the calf may be seen along the isoelectric line in the final record. Similar changes in electrocardiographic pattern were found in a tracing obtained three days before the death of Calf E857. A still greater disruption of electrocardiographic pattern was observed in the final record obtained from Calf E778. A suggestion of premature ventricular beats, with a tendency towards coupling, was noted in the final electrocardiogram of this subject. Other characteristics of the final tracing made four days before death included an alteration of the R waves and a widening of the QRS interval. All three of these corn oil filled milk calves were afflicted with symptoms of weakness and muscular involvement (3) at the time final records were obtained.

Calves E851 and E857 had final tocopherol levels of 65 and 175 μg. per 100 ml. of blood plasma, respectively. Cardiac and skeletal muscle lesions were demonstrated in both individuals. White streaks were observed grossly in the left ventricle of Calf E851. Microscopic examination revealed a proliferation of cardiac muscle nuclei and fibrosis in an area taken from the left ventricle. The only remarkable finding in the heart section obtained from E857 was an accumulation of fibroblasts around the inner Purkinje fibers. No data are available regarding the plasma tocopherol values or necropsy findings for Calf E778. The gross changes which occurred in Animals E851 and E857 appear similar to those observed by Bacigalupo *et al.* (5) in tracings taken within 24 hr. preceding the sudden death of vitamin E–deficient lambs.

Dietary factors presumably responsible for the changes found in electrocardiograms of calves fed the corn oil filled milk diet can not be ascertained



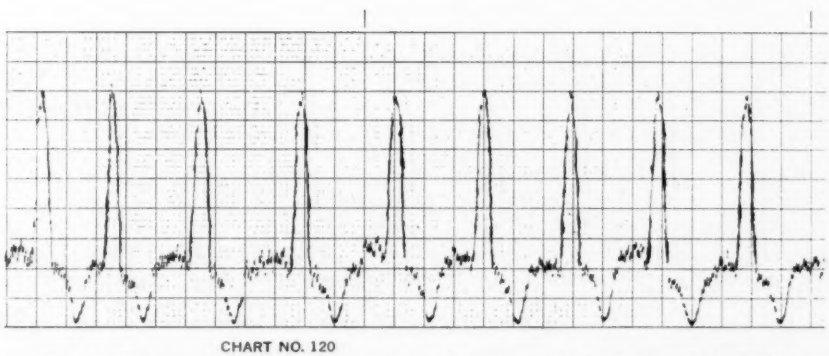


FIG. 6. Electrocardiographic changes in serial tracings obtained from Calf E-851, corn oil filled milk diet. Top, pre-experimental; middle, ten days before death; bottom, two days before death.

from the available data. Likewise, it can not be determined to what extent these changes resulted from the generally poor physical condition of the animals from two to four days prior to death, or were due to actual heart damage. It should be noted that no remarkable changes were observed in electrocardiograms of three additional corn oil and lard filled milk subjects in which cardiac lesions were demonstrated. A similar finding was reported by Bacigalupo *et al.* (5). These workers found that no significant alterations in electrocardiographic records occurred in some tocopherol-deficient lambs showing cardiac lesions upon necropsy.

Creatinuria ratios. An examination of the data pertaining to creatinuria ratios revealed that no marked changes occurred during the experimental period in all groups. The average daily excretion of creatine and creatinine, expressed as milligrams per kilogram of body weight, generally decreased on all diets during the course of the experiment. The absence of creatinuria in calves afflicted with nutritional muscular dystrophy is in agreement with results obtained by contemporary workers (7, 18).

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PASTURE FOR YOUNG DAIRY CALVES[1,2]

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SUMMARY

Two experiments were conducted with 32 dairy calves to study the value of pasture. In the first experiment, 22 Holstein calves were divided into three groups: barn-fed, pasture, and pasture plus hay free choice. The average daily gains were 0.97, 1.14, and 1.10 lb., respectively, to 60 days of age and 1.46, 1.67, and 1.70 lb., respectively, from 60 to 116 days of age. The differences in average daily gain were not statistically significant. The pasture group consumed 98.4 lb. less grain and the pasture-hay group 107.0 lb. less grain than the barn-fed group.

The results in the second experiment were similar to those in the first experiment, except that the Guernsey calves did not respond favorably.

Observations indicated that the pasture-hay calves consumed hay throughout the experiment even when an adequate supply of lush pasture was available. Calves having access to hay had a firmer feces and smoother hair coat than calves not having access to hay. Calves on pasture were observed to ruminate earlier than calves reared in the barn. All fecal samples were negative for parasite ova and coccidial oocysts. There are indications that consideration should be given to pasture mixtures for calves.

Although the rearing of calves on pasture has received considerable attention in New Zealand (7) and England (9), it has attracted little attention in America (6). In America, the usual practice is to wait until the calves are at least 6 mo. old before placing them on pasture. It would appear that this practice is based not upon experimental evidence but upon farmer experiences. Although very little is known of the ability of calves to utilize grass, there is evidence (2, 3, 5) which shows that 8- to 12-wk.-old calves can digest pasture as well as can mature cows. Another report indicates that the 3-wk.-old calf is able to digest pasture grasses as well as can older calves (8). This suggests that stomach capacity rather than digestion is the limiting factor.

Another reason for hesitating to place calves on pasture at an earlier age is their susceptibility to worm parasites. That parasites may not be a problem was suggested by the experiments of McCullough and Sell (6), who reported a low incidence of internal parasitism over a 4-yr. period. Even if parasites are present, it is unlikely that they will cause difficulty as long as an adequate supply of high-quality forage is available. Roy *et al.* (9) found that even when levels of infestation were above the values considered pathogenic for older animals, growth rate was not unduly depressed. In some cases, the best growth rates were obtained with calves showing the highest egg counts.

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[2] The data reported here were taken from the thesis submitted by M. C. Stillions to the Graduate School, Purdue University, in partial fulfillment of the requirements for the Master of Science degree.

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The purpose of these investigations was to study the value and feasibility of using pasture for rearing young dairy calves under Indiana conditions, and to compare the results with calves reared under a conventional system.

EXPERIMENT I

Experimental procedure. In this experiment, 22 Holstein (16 bulls and six heifers) calves were assigned at random to one of three experimental groups: (1) barn-fed, (2) pasture, and (3) pasture-hay. The barn-fed calves were reared in a pole-type bulding with individual pens, feeders, and outside runways. The pasture and pasture-hay calves were placed on a pasture some distance from a barn. A pole-type shed covered with polyethylene plastic was used to provide shelter, and grain was available in individual covered feed boxes.

The pastures were divided into plots and the calves were rotated at intervals to provide them with a succulent, digestible forage at all times. The pasture forage consisted of an alfalfa-ladino-brome mixture at the beginning of the experiment. As the experiment progressed, ladino clover became the predominant forage.

The calves were obtained from the Purdue University and other state institution herds. All calves were left with their dams for variable lengths of time to obtain colostrum, then fed from open pails. All observations began when the calves were taken from their dams. The calves were not placed on pasture until at least eight days of age, but no later than 14 days. The daily feeding schedule is shown in Table 1 and the composition of the grain mixture in Table 2.

All calves were given access to grain three times daily. Hay was available to calves in Group 1 beginning at 15 days of age and to Group 3 when placed on pasture. Calves in Group 2 received no hay. Water was provided ad libitum by the end of the 2nd wk.

When the calves in Groups 2 and 3 were 61 days old, they were moved into larger pasture areas and the grain allowance was reduced to 2 lb. per calf daily until the experiment terminated at 116 days of age. Group 1 calves were kept in

TABLE 1

Daily feeding schedule

Age	Whole milk	Grain[a]	Hay
(days)		*(lb.)*	
0–2	On cow	....	....
3–7	6	....	....
8–28	8	3	Free choice[b]
29–35	6	3	Free choice
36–42	4	3	Free choice
43–49	2	3	Free choice
50–60	....	4	Free choice
61–116	....	2[c]	Free choice

[a] Indicates maximum allowed daily.

[b] Hay feeding began at 15 days of age for Group 1 calves and when placed on pasture for calves in Group 3. No hay was fed to calves in Group 2.

[c] Group 1 calves were continued on a maximum of 4 lb. grain daily to 90 days of age, then reduced to 2 lb. daily until the experiment was terminated.

TABLE 2

Composition of grain mixture

Ingredient	Per cent
Ground corn	58.0
Ground oats	25.0
Soybean oilmeal	15.0
Steamed bonemeal	1.0
Iodized salt	1.0
Total	100.0
Per cent protein	15.0

individual pens and allowed a maximum of 4 lb. grain daily until 90 days of age. They were then placed in large pens and group-fed 2 lb. grain daily and hay free choice until the experiment terminated. Calves in all groups were given free access to a mixture made up of two parts steamed bonemeal and one part iodized salt.

The calves were weighed at the beginning of the experiment and twice weekly until 60 days of age, then every other week until the end of the experiment.

Regular observations were made of the health and condition of the calves and, specifically, the incidence of diarrhea. Individual fecal samples were taken at the beginning and every 2 wk. during the experiment. Fecal egg counts for parasites were made according to the method of Stoll (10).

Results. The average daily gains for the calves to 60 days of age (Table 3) were 0.97, 1.14, and 1.10 lb., respectively, for the barn-fed, pasture, and pasture-hay calves. From 60 to 116 days of age the three groups of calves gained an average of 1.46, 1.67, and 1.70 lb., respectively (Table 4). Analyses of variance showed that in weight gains the differences were not statistically significant. Although the barn-fed calves made slower weight gains than either of the pasture groups, even these calves compared favorably to Holstein calves in the USDA Standard (Figure 1).

Feed consumption data are presented in Tables 3 and 4. The differences in amount of whole milk consumed are due to differences in age when calves were placed on experiment. Analysis of variance of the data in Table 3 showed that to 60 days there was a significant difference in grain intake between the three

TABLE 3

Average weight gains and feed consumption to 60 days of age

Calves	Barn-fed	Pasture	Pasture-hay
Number	7	7	8
Days on experiment	51.3	53.0	53.6
Days on pasture	.....	48.9	50.1
Growth rate			
Initial weight *(lb.)*	99.3	93.3	96.9
Average daily gain *(lb.)*	0.97	1.14	1.10
Standard error of mean *(lb.)*	.05	.07	.31
Feed consumption			
Whole milk *(lb.)*	236.0	247.7	251.0
Grain *(lb.)*	64.5	43.4	36.9
Hay *(lb.)*	44.5	.....	10.0

TABLE 4

Average weight gains and feed consumption from 60 to 116 days of age

Calves	Barn-fed	Pasture	Pasture-hay
Number	7	7	8
Growth rate			
Initial weight *(lb.)*	148.7	153.1	155.8
Average daily gain *(lb.)*	1.46	1.67	1.70
Standard error of mean *(lb.)*	.06	.28	.42
Feed consumption			
Grain *(lb.)*	187.3	110.0	107.9
Hay *(lb.)*	256.2	.....	20.5

groups. This occurred even though the amount of grain allowed was the same for all groups. The pasture group consumed an average of 21.1 lb. less grain and the pasture-hay group 27.6 lb. less grain than the barn-fed group. It was, likewise, noted that the average grain intake of calves in the pasture group varied from 22 to 74 lb. and from 16 to 69 lb. for the pasture-hay group. From 60 to 116 days (Table 4), the average grain consumptions were 187.3, 110.0, and 107.9 lb., respectively, for the three groups.

The pasture-hay calves consumed an average of 10.0 lb. hay to 60 days and 20.5 lb. from 60 to 116 days of age, as compared to 44.5 and 256.2 lb. by the barn-fed calves for comparable periods. This intake of hay by the pasture calves occurred even though young, lush pasture was readily available.

The pasture-hay calves produced a firmer feces and had a smoother hair coat than the pasture calves not receiving hay. The value of hay as an aid in the prevention of laxative feces was particularly noticeable when ladino clover was the predominating forage. At the conclusion of the experiment, the

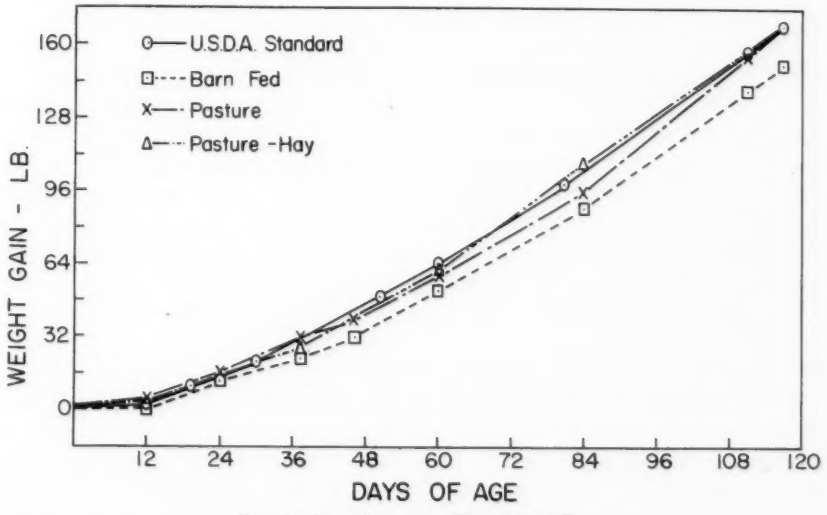

FIG. 1. Growth curves, Experiment I.

general appearance of the pasture-hay group was superior to the other groups, followed in order by the pasture and barn-fed groups.

Microscopic examinations of individual fecal samples for parasite ova and coccidal oocysts were negative throughout the experiment.

EXPERIMENT II

Experimental procedure. Six Holstein and four Guernsey calves were assigned to two groups: (1) alfalfa-ladino-brome pasture, and (2) alfalfa-ladino-brome pasture plus hay free choice. All calves were obtained from the Purdue University and other state institution herds.

The procedures used in this investigation were similar to those described in Experiment I, with the noted modifications. The daily feeding schedule (Table 5) allowed less whole milk than in Experiment I. It was necessary to

TABLE 5

Daily feeding schedule

Age	Whole milk		Grain[a]	Hay
	Holstein	Guernsey		
(days)	*(lb.)*			
0–2	On cow	On cow	....	....
3–7	6	6	....	....
8–14	8	6	3	....
15–21	6	6	3	Free choice[b]
22–28	4	4	3	Free choice
29–35	2	4	3	Free choice
36–42	2	2	4	Free choice
43–60	....	2	4	Free choice

[a] Indicates maximum allowed daily.
[b] Only Group 2 (pasture-hay) received hay.

modify the milk-feeding schedule for the Guernsey calves, to compensate for their lower body weights and the increase in incidence of diarrhea. Also, the Guernsey calves were fed milk to 60 days of age, as compared to 42 days for the Holsteins.

Results. The average daily gains for the pasture and pasture-hay groups were 0.80 and 0.81 lb., respectively, as shown in Table 6. The average daily gains of the Holstein calves were 0.97 and 1.07 lb., respectively, which is comparable to the gains of the Holstein calves in Experiment I to 60 days of age. In contrast, the Guernsey calves gained 0.55 and 0.41 lb., respectively, for the two groups.

During the experiment, the Holstein calves received an average of 176.0 lb. whole milk compared to an average of 211.5 lb. whole milk for the Guernsey calves. The Guernsey calves consumed an average of 23.5 lb. grain compared to 57.5 lb. by the Holstein calves. The calves in the pasture-hay group consumed an average of 5.5 lb. hay to 60 days. All fecal samples were negative for parasite ova and coccidial oocysts.

TABLE 6

Average weight gains and feed consumption to 60 days of age

Calves	Pasture	Pasture-hay
Number	5	5
Breed	3 H., 2 G.	3 H., 2 G.
Days on experiment	55.2	57.6
Days on pasture	51.2	51.2
Growth rate		
Initial weight *(lb.)*	81.6	68.4
Average daily gain *(lb.)*	.80	.81
Feed consumption		
Whole milk *(lb.)*	183.6	200.1
Grain *(lb.)*	44.4	43.6
Hay *(lb.)*	.....	5.5

DISCUSSION

That calves can make excellent use of good quality pasture at an early age was demonstrated in these investigations. These findings are contrary to the beliefs commonly held by many individuals, and are contrary to the usual recommendations, but they are in agreement with results reported by other workers (6, 7, 9), which suggest that calves can make excellent use of pasture at an early age.

Analyses of the growth curves in Figure 1 show that the barn-fed calves made consistently slower weight gains than the pasture groups and that the differences in favor of the pasture groups were still present at 116 days of age. The increased weight gains of the pasture groups are in agreement with the results obtained by other workers (6, 7, 9).

Th growth rates of the Holstein calves in Experiment I were comparable to those of Holstein calves in the USDA Standard (4). This is of interest, since the experimental calves received an average of 243.9 lb. whole milk to 50 days of age compared to whole milk until 4 wk. old, followed by skimmilk to 6 mo. of age by the calves as reported in the USDA Standard. That even less whole milk may be required was suggested by the results obtained with the Holstein calves in Experiment II, which consumed an average of 67.9 lb. less whole milk than calves in Experiment I but made comparable weight gains.

The ability of calves to utilize pasture was demonstrated by the pasture groups which made greater average daily gains than the barn-fed calves, with an average of 102.7 lb. less grain. The lower grain consumption was due to grain refusal. However, there was considerable variation between individual calves in grain consumption during the first 60 days. Inspection of the data indicated that there was no apparent correlation between grain consumption and weight gains, which suggests that there was considerable variation between calves in the amount of nutrients obtained from pasture. The increased grain consumption of the calves in Experiment II may have been the result of the lower amount of whole milk fed, animal variation, or the lower quality and quantity of the pasture during the experiment. Observations made during the experiment indicate that the poor results obtained with the Guernsey calves

in Experiment II were a reflection of their low grain consumption, or their unwillingness to consume sufficient pasture to overcome their lack of grain consumption.

Pasture consumption was observed to occur almost immediately when the calves were placed on pasture. One eight-day-old calf began consuming pasture immediately and was ruminating within 4 hr. after being placed on pasture. In general, calves on pasture began ruminating at a much earlier age than calves reared in the barn. At the present time there appears to be no satisfactory explanation or agreement as to when calves start ruminating. Reports range from as early as six days under barn feeding conditions (4) and seven days on pasture (7), to an average of 28.7 days under a barn-feeding system (1). Our observations are in close agreement with McMeekan (7) for calves reared on pasture and Ackerman *et al.* (1) for calves reared in the barn. The earlier observed rumination by calves on pasture may be the result of pasture consumption and its stimulatory effect on rumen activity. In a recent report, Preston *et al.* (8) suggested that the ability of the calf to ferment and digest grass dry matter was almost instantaneous and was dependent only on there being a suitable substrate in the rumen.

Observations made during both experiments suggest that calves on pasture have a desire for some coarse material which may be supplied as hay or pasture. Calves appeared to prefer brome grass, even when it was fairly mature, followed by alfalfa and then ladino clover. Ladino clover did not appear to be very palatable to young calves. Also, its consumption appeared to result in the production of a softer feces. The value of a dry feed for calves on pasture was indicated by the firmer feces and improved general appearance of the pasture-hay calves. The desire of calves for a dry feed while on lush pasture was also noted by Roy *et al.* (9).

Although it is recommended that young calves not be pastured because of parasites, the parasite counts were negative throughout both of these experiments. This suggests that the locations were free from parasites. Fecal samples taken from growing and adult animals in the Purdue University herd were also negative for parasites. It would appear that individual herds may be kept free of parasites if precautions are taken in bringing outside animals into the herd. It was suggested by Roy *et al.* (9) that even if parasites are present they may not affect the calf unduly, provided the pasture is plentiful and of good quality.

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THE STIMULATORY EFFECT OF SODIUM BUTYRATE AND SODIUM PROPIONATE ON THE DEVELOPMENT OF RUMEN MUCOSA IN THE YOUNG CALF

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SUMMARY

Twelve young dairy calves equipped with rumen cannulae were fed milk and housed in tie stalls with elevated floors to prevent bedding consumption. Sodium acetate, sodium propionate, sodium butyrate, sodium chloride, and glucose were administered over an 11-wk. period, to study their effect on rumen mucosal development. Two calves served as controls. Administration of solutions of sodium butyrate or sodium propionate caused marked development of the rumen mucosa, whereas the other materials caused relatively little growth. It is hypothesized that the growth of rumen papillae is probably a result of the metabolism of certain compounds by the rumen wall and/or their effect on blood flow in the rumen.

The newborn ruminant has a forestomach that is rudimentary both anatomically and physiologically. Contrary to early beliefs, the stimulus necessary to develop the forestomach tissues of young ruminants does not come entirely from the physical nature of the feed. Flatt *et al.* (7) reported that a low-fiber purified diet or a solution of the mixed sodium salts of acetic, propionic, butyric, and lactic acids caused the development of rumen papillae; whereas, coarse, inert materials, such as plastic sponges, were ineffective. The biochemical or physiological basis for the growth of rumen papillae following contact with the volatile fatty acids or a fermenting low-fiber diet is not clear.

In these experiments, stimulation of papillary growth using several pure, absorbable, and/or metabolizable compounds was measured.

EXPERIMENTAL PROCEDURE

Experimental animals. Twelve grade Holstein bull calves purchased at local auctions were fitted with cannula-type rumen fistulae (inside diameter ¾ in.) at 2 to 5 wk. of age, according to the method of Dougherty (4). They were confined in tie stalls equipped with elevated wooden platforms to prevent bedding consumption, and fed whole milk from nipple pails. The amount of milk fed was adjusted periodically in an attempt to keep the rate of gain uniform. Iodized salt and water were available free-choice. A mineral supplement and vitamin D were provided as previously reported (Warner *et al.*, 19). The calves were weighed weekly and records of feed consumption were kept.

Treatments. In addition to the basal milk allotment, eight of the calves were given a solution of either sodium acetate, propionate, butyrate, or chloride; and two were given a glucose solution via rumen cannulae. Two calves were randomly assigned to each treatment and two control calves received only milk.

Stock solutions of the sodium salts of the fatty acids were prepared, using analytical-grade reagents (Table 1), so that one liter of the solution contained an

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TABLE 1
Intakes, weight gains, and age at slaughter of the experimental animals

Calf No.	Treatment[a]	Age at slaughter	Total wt. gain	Total intake of		
				Milk	Test materials[b]	Test materials[b]
		(days)	*(kg.)*	*(kg.)*	*(kg.)*	*(M)*
3	Na Acetate[c]	109	34	586	15.4	257
4	Na Acetate	95	14	454	12.2	203
11	Na Propionate[d]	73	7	279	6.4	86
12	Na Propionate	102	34	516	16.0	216
7	Na Butyrate[e]	79	18	343	7.5	85
8	Na Butyrate	108	36	545	18.4	209
5	Glucose[f]	102	39	492	10.6	59
6	Glucose	102	36	518	10.1	56
9	Na Chloride	101	28	519	12.8	219
10	Na Chloride	103	41	595	8.8	150
1	Control	103	35	550	.....	.....
2	Control	104	41	529	.....	.....

[a] All calves received the test materials for 11 wk., except Calves 4, 11, and 7. These calves died of pneumonia at the 9th, 5th, and 7th wk. of the experimental period, respectively.
[b] Amounts of sodium salts of fatty acids expressed as the acid.
[c] Analytical grade sodium acetate. Mallinckrodt Chemical Works, N. Y., N. Y.
[d] Sodium propionate N.F., E. I. duPont de Nemours & Co., Wilmington, Delaware.
[e] Analytical grade sodium butyrate. Amend Drug Co., N. Y., N. Y.
[f] Cerelose (dextrose). Corn Products Sales Co., Syracuse, N. Y.

amount of the salt equivalent to 250 g. of the respective fatty acid. These stock solutions were reduced to pH 6.7, using concentrated HCl. Before administration, each stock solution was diluted with warm water so that its concentration never exceeded 100 g. of free fatty acid per liter of solution. The amount of fatty acid salt administered was increased gradually during the experimental period.

A stock glucose solution was prepared at a concentration of 50% by weight. The stock solution was administered so that each calf on this treatment received 1.0 g. of glucose per pound of body weight per day.

The sodium chloride solution was slowly infused into the rumen during an 8-hr. period each day, according to Flatt's (8) method. This procedure was employed because typical symptoms of sodium chloride toxicity (Pistor *et al.*, 15), including severe tremors and incoordination in the rear quarters, were seen immediately after 3 liters of a 2% sodium chloride solution were poured into the rumen of a 90-lb. calf. The concentration of the infused solution was gradually increased from 0.9 to 2.4% during the 11-wk. experimental period.

The solutions (sodium chloride excepted) were poured into the rumens twice each day at the time of feeding. Table 1 summarizes the amount of each compound administered during the experimental period.

At the end of the experimental period, the calves were slaughtered. The digestive tracts were removed and the volume of water contained by the reticulo-rumen, filled to an overpressure of 1.5 in. of water, was determined in a manner similar to that used by Warner *et al.* (19). The reticulo-rumens were photographed under standardized conditions and the resulting photographs were used to give each rumen a papillary score, according to the technique of Flatt (8). A

4-in. square of the ventral portion of the dorsal cranial sac of the rumen was removed and manually separated into mucosa and muscle layers. These separated portions were dried at 90° C., allowed to come to equilibrium with the atmosphere, and weighed. The percentage of mucosa was then calculated on an air-dry basis. Following the removal of the 4-in. square of tissue, the rest of the rumen tissue was separated into mucosa and muscle portions. These portions, along with the reticulum and omasum, were dried in a Stokes freeze drier for 24-48 hr., using a temperature of 110° F. The dried tissues were equilibrated with the atmosphere, analyzed for fat-free dry matter, and expressed as grams of fat-free dry matter per unit of ingesta-free body weight (Table 2).

RESULTS AND DISCUSSION

The data obtained during the experimental period are presented in Table 1. All of the calves, except those that died from pneumonia, grew moderately well, as evidenced by the weight gains (Table 1).

The various treatments employed caused marked differences in rumen papillary development. The percentage of air-dry mucosa, the fat-free dry weight

TABLE 2
Data obtained after slaughtering the experimental calves[a]

Calf No.	Treatment	Body wt. (ingesta-free)	Rumen: Papillary score	Rumen: Mucosa[b]	Fat-free dry matter[c]: Rumen: Mucosa	Rumen: Muscle	Total tissue of: Reticulum	Total tissue of: Omasum	Reticulo-rumen volume[c]
		(kg.)		*(%)*	*(g.)*				*(liter)*
3	Na Acetate	74	3	50	73	63	32	38	15
4	Na Acetate	56	3	45	69	96	38	34	24
	Av. (A)			48	71	80	35	36	20
11	Na Propionate	53	5	57	70	78	43	31	....
12	Na Propionate	75	5	62	89	80	40	25	17
	Av. (P)			60	80	79	42	28	....
7	Na Butyrate	58	6	58	84	85	42	36	33
8	Na Butyrate	76	6	73	124	67	48	65	30
	Av. (B)			66	104	76	45	50	32
5	Glucose	80	4	37	42	44	22	35	17
6	Glucose	77	3	31	36	57	24	30	18
	Av. (G)			34	39	50	23	32	18
9	Na Chloride	68	2	40	38	69	24	21	23
10	Na Chloride	78	3	48	46	68	30	38	16
	Av. (S)			44	42	68	27	29	20
1	Control	79	1	30	24	40	17	17	17
2	Control	75	1	34	26	42	18	30	16
	Av. (C)			32	25	41	18	24	16
				B>A,S, G, C P>A,G,C A>C	B,P>S, G,C A>C	N.S.	B>A,S, G,C P>S,G,C A>G,C S,G>C	N.S.	

[a] Data were subjected to an analysis of variance (16) and a multiple range test (5). All significant differences are at the 5% level of probability.
[b] Determined from a 4-in. square removed from the ventral portion of the dorsal cranial sac (air dry basis).
[c] Calculated to 100 kg. of ingesta-free body weight.

of rumen mucosa per unit of body weight, and the papillary rating were used as indices of mucosal growth. Figure 1 shows typical photographs used to arrive at the papillary rating for each rumen. Sodium butyrate and sodium propionate stimulated much more rumen papillary growth than did sodium acetate, sodium chloride, glucose, or the controls. The two aforementioned compounds gave extensive papillary development over a large area of the rumen, whereas sodium acetate, sodium chloride, and glucose gave very limited development only in the ventral part of the dorsal cranial sac, as did the mixture of volatile fatty acids employed by Flatt *et al.* (7). Sodium butyrate and sodium propionate also tended to stimulate more reticulum growth than did the other compounds tested. No statistically significant differences ($P < 0.05$) could be detected between any treatments in terms of rumen muscle or omasal growth.

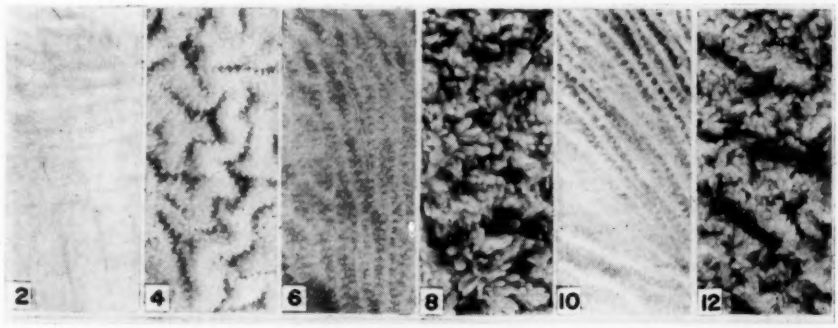

FIG. 1. Photographs of the ventral-most portion of the dorsal cranial sac, just caudal to the rumino-reticular fold. Photograph 2, control; 4, Na acetate; 6, glucose; 8, Na butyrate; 10, Na chloride; and 12, Na propionate. Papillary rating assigned to each photograph found in Table 2.

The volumes of water contained by the filled reticulo-rumens of the calves given sodium butyrate were decidedly larger than those of the calves on the other treatments, which were not appreciably affected by treatment. The physiological basis for this volume increase is not clear from the data obtained in this study.

Since the presence of absorbable fatty acids in the rumen has been shown to stimulate rumen papillary growth (7), it would appear reasonable to predict that the amount of rumen mucosal growth would be related to the degree of physiological or biochemical activity of the compounds as they passed through the membranes of the forestomach, viz., absorption, metabolism, and increased blood flow.

An examination of the literature does not reveal any studies which have duplicated the conditions of this experiment sufficiently to give an unequivocal explanation for the responses observed.

Much confusion exists in the literature that deals with the relative rates at which the volatile fatty acids penetrate the rumen wall (1, 2, 9, 13, 18). The

differences in relative rates of penetration as reported in the literature are not large and might well reflect differences in experimental technique. In studies with normal, conscious animals, in which the problem of interconversion of administered fatty acids within the rumen ingesta is eliminated, it has been shown that no preferental penetration of the fatty acids occurs on a molar basis (6, 17). Thus, one might expect the stimulation of forestomach mucosal growth to be similar for equimolar amounts of the fatty acids tested. As reported in this study, this does not appear to be the case. Calves 7 and 11, which succumbed to pneumonia before the completion of the study, had received approximately 85 *M* of butyric and propionic acid, respectively. Still, each of these calves showed much greater papillary growth (Table 2) than did either of the calves which had received more than 200 *M* of acetic acid.

Pennington (10) and Pennington and Pfander (11) have shown clearly that the butyric, propionic, and acetic acids are actively metabolized by the rumen epithelium in vitro. The relative degree of butyric acid metabolism was greater than it was for either propionic or acetic acids. In the experiment reported here, sodium butyrate afforded the greatest stimulation of rumen mucosa, while sodium acetate produced much less. The mucosal stimulation produced by sodium propionate was slightly less than butyrate, but it was much greater than that produced by acetate. Glucose has also been shown to be metabolized by rumen epithelium (12). If an active metabolism of materials from the rumen is necessary for papillary development, then glucose should have produced more than limited development. Whether the level of administration in this study was too low (Table 1) must be determined by further study.

Dobson and Phillipson (3) reported that increased fatty acid concentrations in the rumen caused increased blood flow from the rumen. Phillipson further reported (14) that the relative rate of increase was butyrate > propionate > acetate. The specific effects of an increased blood flow are difficult to predict, but it could certainly be expected to be associated with rapid tissue growth.

The data reported in this study strongly suggest that the growth of rumen papillae is most probably a result of the metabolism of certain compounds by the rumen wall and/or their effect on blood flow. Further research will be required to justify this concept.

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THE BIOLOGICAL HALF-LIFE OF L-THYROXINE AND L-TRIIODOTHYRONINE IN THE BLOOD OF THE DAIRY COW[1,2]

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SUMMARY

The biological half-life of thyroxine (t ½) is defined as the time in days for one-half of the biological activity of the hormone to be lost. An alternative measurement is the percentage turnover rate per day. By the injection of thyroxine-I^{131} into 16 dairy cows in which recycling of I^{131} was blocked by a goitrogen (thiouracil), the determination of the rate of disappearance of radioactivity from the blood permitted the calculation of t ½ and turnover rate. In 1957, during a period of average daily temperature of 47° F., the mean t ½ was 2.54 days and a turnover rate of 27.6% per day was observed. During a period of average daily temperature of 71° F., the mean t ½ was 2.65 days and a turnover rate of 26.7% was observed. In 1958, measurements were made at these two temperatures. Since these means did not differ significantly, it was concluded that seasonal variation in temperature did not influence thyroxine t ½ or turnover rate. The mean t ½ of 16 observations was 2.47 days and turnover rate 28.4% per day. No breed difference in t ½ in Jersey and Guernsey cows was observed. A significantly faster t ½ of 1.99 days and turnover rate of 35.3% was observed in a study of triiodothyronine. These data indicate that there is a build-up of thyroxine in the blood and total body space significantly higher than the daily thyroxine secretion rate.

A method for the estimation of the daily thyroxine secretion rate of dairy cattle has been described (3), and data have been presented concerning the range and mean secretion rate of cattle during various seasons of the year (5). It was shown that during the summer months in Missouri the secretion rate was reduced to about one-third the rate prevailing during the winter.

In the method proposed, it was pointed out that thyroxine and triiodothyronine as secreted are metabolized in the somatic cells of the body (muscle, brain, liver, and kidney) in which there is a further deiodination of the thyroxine molecule to triiodothyronine and other metabolic products. In the process of metabolism of thyroxine, the removal of one iodine molecule results in the formation of triiodothyronine, a biologically active compound equal to or greater than thyroxine (1). It has been suggested, in fact, that triiodothyronine is the biologically active compound at the cell level. The further metabolism (deiodination) of triiodothyronine results in biologically inactive compounds.

The iodine released is in the iodide form. It can be picked up by the thyroid gland or secreted in the urine. Thyroxine in the blood is also secreted in the bile and discharged into the digestive tract. Part of the thyroxine may be reabsorbed or excreted in the feces.

The rate of disappearance of thyroxine and triiodothyronine from the blood

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by deiodination or excretion is of great physiological importance, because it determines the duration of biological activity of these compounds in the animal body.

If the rate of disappearance were very rapid, the thyroxine secreted each day would be lost each day. The thyroxine secretion rate would then represent the effective blood thyroxine level. If, however, only one-half, one-third, or one-fourth of the thyroxine secreted each day were eliminated each day, then the residual biologically active thyroxine or triiodothyronine would accumulate in the blood and tissues and the biologically effective pool in the body would be proportionately greater than the daily thyroxine secretion rate.

It is now possible to study the rate of deiodination and elimination of the thyroid hormones by the determination of the rate of disappearance of I^{131} from the blood when 1-thyroxine-I^{131} or 1-triiodothyronine-I^{131} is injected into cattle. As the hormones are deiodinated in the tissues, the inorganic I^{131} returns to the blood and then could be reutilized by the thyroid gland or excreted in the urine. Since the reutilization of I^{131} would confuse the picture, it is necessary to block thyroid uptake by the use of a goitrogen.

By this technique, it is possible to determine the rate of deiodination and excretion of thyroid hormones. The biological half-life of a hormone may be defined as the time (in hours or days) required for one-half of the activity to be lost. An alternative measurement is to indicate the percentage turnover rate per hour or day. It indicates what percentage of the hormone secreted per day is metabolized and excreted each day.

The object of this report is to present data on the rate of deiodination and excretion of 1-thyroxine and 1-triiodothyronine in mature dairy cattle and to show the lack of effect of season (environmental temperature) upon the process.

EXPERIMENTAL PROCEDURE

Mature Guernsey and Jersey cows were maintained in a dry lot under uniform nutritional conditions throughout the year with free access to an iodized salt block. At the beginning of each experiment, the cows were injected intravenously (jugular vein) with 200 to 400 μc. of carrier-free 1-thyroxine-I^{131} or 1-triiodothyronine-I^{131}. To prevent thyroid uptake of the metabolized I^{131}, 24 g. of thiouracil was administered daily in a gelatin capsule (4). Blood samples were taken from the jugular vein each day following the injection of the hormone. Measurements of the radioactivity of the plasma were made in a National Radaic scintillation well counter (Model SA-2D). Conventional corrections were made for decay of the isotope and for background.

The residual radioactivity present in the plasma of each cow daily, when plotted on semilog paper, was found to form a straight line indicating the exponential nature of the rate of elimination of the thyroid hormones. The disappearance of radioactivity from the blood is assumed to represent the amount of thyroxine-I^{131} which has been excreted or deiodinated and the iodide-I^{131} formed excreted by the kidneys, since pickup of metabolized I^{131} by the thyroid gland was prevented by the use of a goitrogen.

The plot of the disappearance of I^{131} from the plasma was extrapolated back to zero time (time of injection) and biological half-time (t ½) of thyroxine was determined graphically (Figure 1) or from the slope of the regression line; t ½ represents the time required by the animal body to eliminate one-half of the thyroxine in the circulation.

The rate of metabolism of thyroxine per day was computed from the t ½ estimations by the following equation:

$$\text{Turnover rate per day } (\%) = \frac{100 \times ln_2}{t\ ½}$$

where ln is the natural logarithm.

RESULTS

To determine the rate of disappearance of thyroxine-I^{131} from the blood of dairy cattle, four groups of four cows each were studied during a period of 2 yr. The first study conducted in March, 1957, during a period of average daily temperature of 47° F., showed a mean t ½ of 2.54 days and a turnover rate of 27.6% per day (Table 1). In September, 1957, during a period of average daily temperature of 71° F., the mean t ½ of 2.65 days and a turnover rate of 26.7% was observed. The difference in the mean t ½ of these observations was not significant.

In March, 1958, during a period of average daily temperature of 53° F., a mean t ½ of 2.31 days and a turnover rate of 30.1% was observed; whereas, in

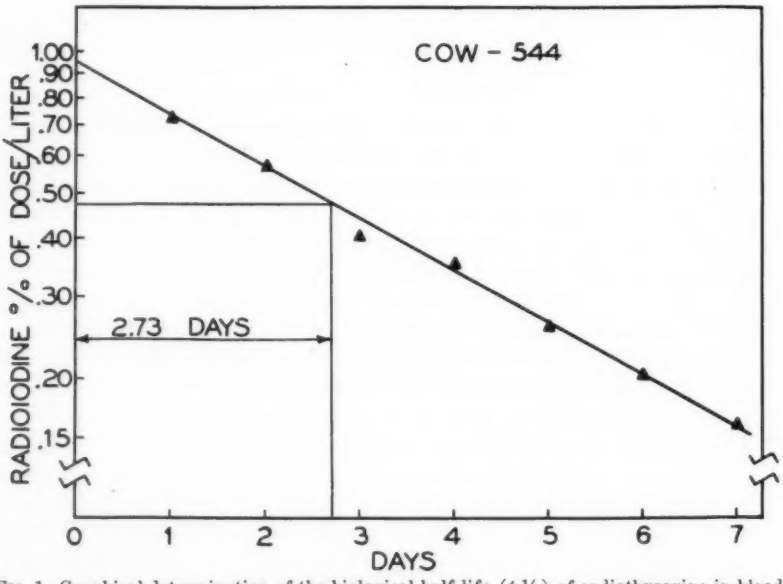

FIG. 1. Graphical determination of the biological half-life (t ½) of radiothyroxine in blood.

TABLE 1

The t ½ and turnover rate of L-thyroxine and L-triiodothyronine in the dairy cow

Breed	Cow No.	Date	Hormone	t½ Days S.E.	Turnover rate (% per day) S.E.	Daily temperature mean
						(° F.)
Guernsey	41	March, 1957	l-thyroxine	2.33 ± 0.053	30.0 ± 1.00	
Jersey	544	March, 1957	l-thyroxine	2.71 ± 0.033	25.4 ± 0.62	47
Jersey	G41	March, 1957	l-thyroxine	2.25 ± 0.070	31.5 ± 1.32	47
Guernsey	38	March, 1957	l-thyroxine	2.87 ± 0.049	23.6 ± 1.03	47
Mean		March, 1957	l-thyroxine	2.54 ± 0.173(1)	27.6 ± 1.86	47
Guernsey	41	Sept., 1957	l-thyroxine	2.45 ± 0.152	28.3 ± 2.87	47
Jersey	544	Sept., 1957	l-thyroxine	3.21 ± 0.070	21.6 ± 1.32	71
Jersey	G41	Sept., 1957	l-thyroxine	2.67 ± 0.028	25.9 ± 0.65	71
Guernsey	38	Sept., 1957	l-thyroxine	2.25 ± 0.026	30.8 ± 0.51	71
Mean		Sept., 1957	l-thyroxine	2.65 ± 0.206(2)	26.7 ± 1.95	71
Guernsey	38	March, 1958	l-triiodothyronine	1.83 ± 0.105	37.9 ± 2.53	53
Jersey	158	March, 1958	l-triiodothyronine	1.88 ± 0.070	36.9 ± 1.67	53
Jersey	160	March, 1958	l-triiodothyronine	2.10 ± 0.065	33.3 ± 1.55	53
Guernsey	3596	March, 1958	l-triiodothyronine	2.14 ± 0.168	33.2 ± 4.03	53
Mean		March, 1958	l-triiodothyronine	1.99 ± 0.077(3)[a]	35.3 ± 1.22	53
Jersey	348	March, 1958	l-thyroxine	2.43 ± 0.069	28.6 ± 1.13	53
Jersey	580	March, 1958	l-thyroxine	2.10 ± 0.068	33.5 ± 1.29	53
Jersey	594	March, 1958	l-thyroxine	2.42 ± 0.061	28.7 ± 1.16	53
Jersey	601	March, 1958	l-thyroxine	2.30 ± 0.052	29.6 ± 1.00	53
Mean		March, 1958	l-thyroxine	2.31 ± 0.082(4)[a]	30.1 ± 1.15	53
Jersey	G41	July, 1958	l-thyroxine	2.49 ± 0.440	27.9 ± 10.52	73
Guernsey	38	July, 1958	l-thyroxine	2.69 ± 0.261	25.8 ± 6.24	73
Guernsey	41	July, 1958	l-thyroxine	1.97 ± 0.124	35.3 ± 2.98	73
Jersey	348	July, 1958	l-thyroxine	2.49 ± 0.090	27.8 ± 2.34	73
Mean		July, 1958	l-thyroxine	2.41 ± 0.229(5)	29.2 ± 5.52	73

[a] Difference of mean (3) and (4) significant at $p < .01$; other means not significantly different.

July, with average daily temperature of 73° F., the $t\ ½$ of 2.41 days and a turnover rate of 29.2% was shown. The difference in the mean $t\ ½$ of these observations was also not significant. From these observations it may be concluded that the rate of disappearance of thyroxine-I^{131} from the blood of mature dairy cattle is not significantly influenced by seasonal variation in temperature at a time when thyroxine secretion rate is markedly influenced.

Since seasonal temperature did not influence $t\ ½$ of thyroxine, the 16 observations were combined. The mean $t\ ½$ was 2.47 days, with a mean turnover rate of 28.4%. Ten measurements on Jerseys showed a mean $t\ ½$ of 2.50 days, with a mean turnover rate of 28.0%; whereas, six measurements on Guernseys showed a mean $t\ ½$ of 2.42 days and a mean turnover rate of 28.9%. No breed difference between $t\ ½$ in Jerseys and Guernseys is indicated.

Triiodothyronine has been shown to be secreted by the thyroid gland in small amounts and occurs in the blood in increased amounts as a result of the first step in deiodination of thyroxine. Four cows were injected with triiodothyronine-I^{131} during March, 1958, at the same time that the group was injected with thyroxine. This group showed a mean $t\ ½$ of 1.99 days and a turnover rate of 35.3%. The difference in means of $t\ ½$ between thyroxine and triiodothyronine is significant at $p < 0.01$.

DISCUSSION

The rate of metabolism and excretion of hormones is of great significance in the animal body for it measures the duration of effectiveness of the hormone upon the cells of the target organ or tissue which it influences. If the hormone is quickly metabolized (a few minutes) and eliminated as appears to be true of the steroid hormones (2), then the hormone blood level would be dependent upon hormone secretion rate from hour to hour. In the present study, it was shown that the mean $t\ ½$ of thyroxine was 2.47 days, or that 28.4% of the injected hormone was removed from the blood each day. This means that the thyroxine secreted by a cow per day required 2.47 days for one-half of it to be eliminated from the blood and 2.47 days for one-half of the remainder. As a result of the relatively slow metabolism and excretion of thyroxine shown by this study, it appears that there is a build-up of thyroxine in the blood and total body space significantly higher than the daily thyroxine secretion rate. Using $t\ ½$ of 2.5 days and turnover rate of 28.0%, it is possible to calculate the total blood and body thyroxine pool in animals secreting various levels of thyroxine per day by the equation:

$$E = \frac{X(1 - K)}{K}$$

Where E is the total blood and body thyroxine pool, X is the daily thyroxine secretion rate, and K is the percentage turnover rate for thyroxine. Thus, in cattle secreting 5, 10, or 15 mg. of thyroxine per day/1,000 lb. body weight, the corresponding blood and body thyroxine pools would be 12.7, 25.5, and 38.5 mg., respectively (Table 2). The body thyroxine pool would be in equilibrium during periods of constant daily thyroxine secretion. However, with changing thyroxine

TABLE 2

Thyroxine secretion rate, blood and body thyroxine pool, and effect of replacement therapy and stoppage in cattle

Daily thyroxine secretion rate/1,000 lb. body weight	Effective body thyroxine pool[a]	Thyroprotein replacement 15.0 g/day	
		Days to reach apparent equilibrium[b]	Withdrawal days to return to normal level[c]
(mg.)	*(mg.)*		
2	5.1	23	7.2
4	10.2	22	4.7
5	12.7	22	3.9
6	15.4	22	3.2
8	20.4	20	2.2
10	25.5	19	1.4
12	30.8	17	0.78
15	38.6	.....	.....

[a] Calculated by formula $E = \frac{X(1-K)}{K}$ (see page 1610)

[b] Calculated by assuming a constant 28% turnover of thyroxine in the body.

[c] Calculated by the equation $E_n = E_o e^{-kt}$ where E_n = new elevated equilibrium of blood and body thyroxine pool under thyroprotein therapy; E_o = original endogenous equilibrium of total blood and body thyroxine pool; K = turnover % daily of thyroxine in body; t = time in days; e = base of natural logarithms.

secretion rate with temperature changes, the body thyroxine pool would change correspondingly. It will be noted, however, that the rise in the body thyroxine pool is slow during periods of increasing thyroxine secretion, whereas the decline is rapid during periods of decreasing thyroxine secretion.

This change can be shown in cows when replacement therapy with thyroprotein is practiced. The recommended level of thyroprotein-feeding is 15 g. per day to 1,000-lb. cows. It has been observed in our laboratory that 1 g. of thyroprotein fed is equal to the subcutaneous injection of 1 mg. of thyroxine. Thus, animals fed 15 g. of thyroprotein per day, whose daily thyroxine secretion rate was 5 mg. per day/1,000 lb. body weight, would require 22[3] days to reach apparent equilibrium of effective body thyroxine pool of 38.6 mg., which would be equal to the body pool of a cow secreting 15 mg. per day (Figure 2). On the other hand, upon the complete withdrawal of thyroprotein, the cow would return to the previous body pool of 12.7 mg. in 3.9 days. Similarly, cows secreting 6 mg. per day would return to the normal level in 3.2 days. The rapidity of decline is due to the destruction of the hormone at a constant rate while there is no endogenous secretion, since the thyroid gland is completely inactive during this period. In the case of cows fed thyroprotein for an extended period, the thyrotropin-secreting cells of the pituitary cease secretion and the thyroid gland would stop iodine accumulation and secretion of thyroxine. Upon the sudden withdrawal of thyroprotein and the rapid drop in the blood and body thyroxine pool, there would be a time lag in the resumption of TSH-thyroxine secretion for several days. In lactating cows this is shown by the precipitous drop in milk yield.

[3] Theoretically, equilibrium would be reached only at infinity. However, the magnitude of change after attaining 99% equilibrium is very small and if 99% of effective body and blood thyroxine equilibrium level is considered satisfactory, it will then take 13 days to reach equilibrium.

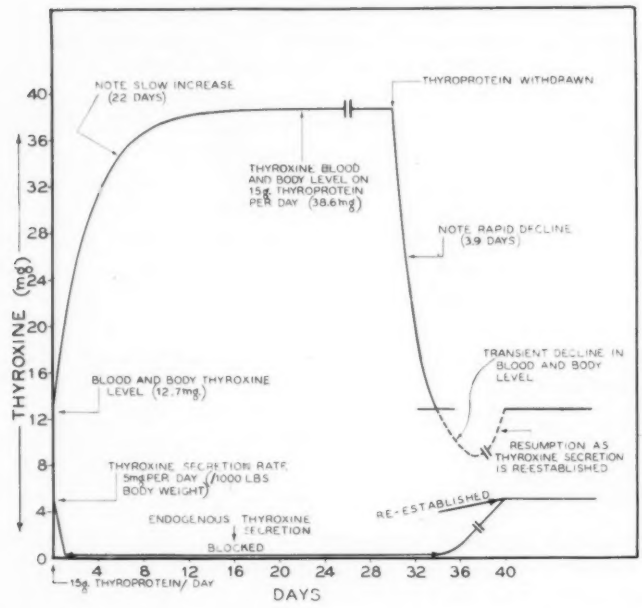
NOTE SLOW INCREASE (22 DAYS)
THYROPROTEIN WITHDRAWN
THYROXINE BLOOD AND BODY LEVEL ON 15g. THYROPROTEIN PER DAY (38.6mg.)
NOTE RAPID DECLINE (3.9 DAYS)
TRANSIENT DECLINE IN BLOOD AND BODY LEVEL
BLOOD AND BODY THYROXINE LEVEL (12.7mg.)
RESUMPTION AS THYROXINE SECRETION IS RE-ESTABLISHED
THYROXINE SECRETION RATE 5mg. PER DAY (1000 LBS. BODY WEIGHT)
RE-ESTABLISHED
ENDOGENOUS THYROXINE SECRETION BLOCKED
THYROXINE (mg.)
15g. THYROPROTEIN/ DAY
DAYS

FIG. 2. Effect of feeding thyroprotein and sudden withdrawal on blood and body thyroxine level (pool). When 15 g. thyroprotein is fed to a cow secreting 5 mg. thyroxine per day, whose blood and body level is 12.7 mg., it requires 22 days to reach apparent equilibrium of the elevated effective body level of 38.6 mg. On complete withdrawal of thyroprotein, it requires only 3.9 days for the return to the previous thyroxine body level of 12.7 mg. However, when thyroprotein is fed for an extended period, TSH and thyroxine secretion is inhibited until the body thyroxine pool returns to 12.7 mg. Since time is required to re-initiate thyroxine secretion, the thyroxine body pool declines below normal, causing milk secretion to decline below normal. As TSH-thyroxine secretion is resumed, lactation increases to the normal level.

However, if the withdrawal of thyroprotein is gradual, as suggested by Swanson (6) and Swanson and Hinton (7), there would then be a gradual reduction in the thyroxine pool and time would provide for the gradual resumption of TSH and thyroxine secretion as the normal thyroxine secretion rate was approached.

These data are of significance also in understanding the pituitary thyrotropin–thyroid gland relation in estimating thyroxine secretion rate. As exogenous thyroxine is administered in increasing amounts below the normal thyroxine secretion rate level, the exogenous hormone would push the effective blood and body thyroxine pool above normal. The increased level of blood thyroxine would immediately act upon the TSH secretion rate and lower it slightly (Figure 3). A corresponding reduction in thyroidal-I^{131} discharge would occur. With each increase of exogenous thyroxine there would be a corresponding reduction of TSH and thyroidal-I^{131} release until, at the point of equilibrium of exogenous and

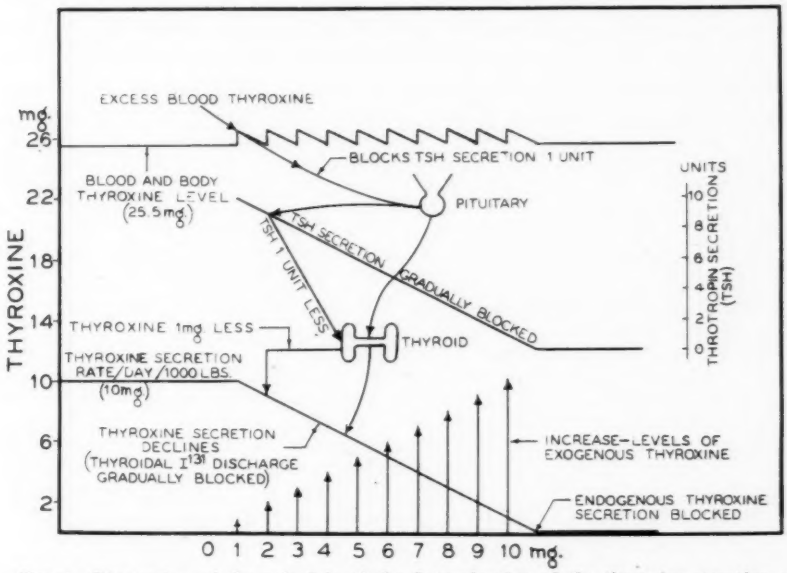

FIG. 3. Illustration of the principle of the determination of the thyroxine secretion rate by replacement therapy in a cow secreting 5 mg. thyroxine per day, with an effective body level of 25.5 mg. thyroxine. As exogenous thyroxine is administered in increasing amounts below the normal thyroxine secretion rate level, the effective blood and body thyroxine pool is elevated (i.e., slightly more than 25.5 mg.), which then acts upon the pituitary gland to reduce thyrotropic hormone secretion (TSH) slightly. A proportional reduction in I^{131} discharge from the thyroid gland will occur. The same phenomena will continue with increasing levels of thyroxine and, at the point where exogenous thyroxine equals endogenous production, all TSH and thyroidal I^{131} discharge will completely stop, indicating the blockage of endogenous thyroxine secretion. The dosage at which these phenomena occur is taken as the normal thyroxine secretion rate.

endogenous thyroxine, TSH and thyroidal-I^{131} release would stop.[4] This level of exogenous thyroxine, then, equals the endogenous thyroxine secretion rate. On the other hand, before this relationship was fully appreciated an injection of thyroxine in excess of the thyroxine secretion rate was administered, then reduced levels of thyroxine were administered. However, in such cases, the blocking dosages required time for their metabolism and elimination as indicated by these data and, as a consequence, the actual thyroxine secretion rate was underestimated, due to the time lag in thyroxine metabolism.

That the metabolism of triiodothyronine should proceed at a faster rate than

[4] It is generally recognized that the thyroid gland has a limited amount of autonomous functional capacity. That is, the thyroid gland may function slightly in the absence of TSH. Thus, slight thyroidal-I^{131} release may occur even after TSH blockage by thyroxine has been produced. The autonomous release of thyroidal-I^{131} seldom exceeds 5 to 10% of the previous count.

thyroxine is understandable, because the former compound has one less iodine molecule subject to deiodination. In spite of this difference in rate of metabolism, triiodothyronine appears to be equal to or slightly more active biologically than thyroxine. However, no direct evidence concerning comparisons of the two compounds in blocking TSH thyroidal-I^{131} release in cattle has been reported.

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TECHNICAL NOTES

METHOD FOR DETERMINING PRODUCT LOSS OF VACUUM-TREATED MILK

Vacuum treatment accompanied by flashing in nonsteam-injection systems for the removal of off-flavors from fluid dairy products results in concentration or loss of product. A comparison of Mojonnier total solids determinations on the original raw and treated milk samples has been used as a means of ascertaining these losses (2). The method is time-consuming and subject to the inherent errors of the Mojonnier test for total solids. Furthermore, the computed daily loss, based on periodic total solids determinations during processing, is not a true indication of the loss, because of variations in degrees of flash-cooling during a daily operation. The need for a practical method for determining product loss is apparent. This paper describes a method for determining product loss when fluid dairy products are treated in nonsteam-injection vacuum equipment.

METHOD

Vapors leaving the vacuum chamber on nonsteam-injection flavor improvement equipment are condensed by a water condenser located in the vapor line ahead of the vacuum pump. This is shown to the right of the broken line in Figure 1. Normally, the condenser water flows to the drain. The proposed method recirculates this water through a water collection system as noted to the left of the broken line in Figure 1. The water leaving the vacuum pump in a 1-in. stainless steel pipe flows through a sweet-water plate cooler which cools the water to approximately 35° F. This chilled water then enters a degassing chamber (located at a height above the vapor line condenser) through a 1-in. perforated stainless steel pipe and returns through ½-in. copper tubing to the vapor line condenser to complete the cycle. The excess water, resulting from condensation of the vapors leaving the vacuum chamber, overflows from the degassing chamber into an overflow cup and then into a condensate receiver on a scale. The weight of water accumulated per unit of time, divided by the pounds of milk processed per unit of time, multiplied by 100, results in the percentage of milk loss.

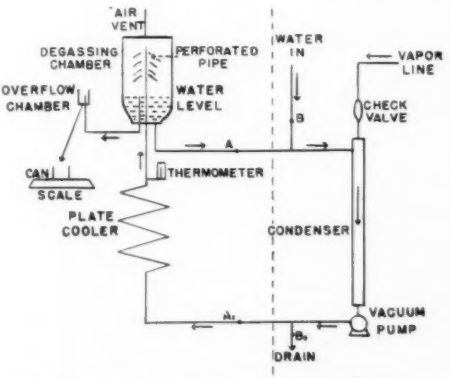

FIG. 1. Water collection system for estimating product loss resulting from vacuum treatment of milk.

The degassing chamber (19 in. by 9 in., tapering to a 4-in. diameter at the bottom) consists of a glass milk-receiver jar with a stainless steel plate at each end which allows for making the pipe line connections. The 1-in. perforated pipe which extends 16½ in. into the degassing chamber has ten sets of four ⅜-in. holes drilled 90° apart on the circumference. Each set of holes is ¾ in. apart, commencing 2 in. below the sealed upper end of the pipe. The air vent (1½-in. stainless steel pipe 6 in. long) prevents possible splash losses of water and pressure build-up by releasing the noncondensable gases.

The water collection system is started by opening Valves A, A_1, and B, closing Valve B_1, and starting the vacuum pump. When the system becomes filled to such an extent that water is flowing from the overflow chamber, Valve B is closed. Time should be allowed for the system to become stabilized so that a uniform overflow of water is established before water collection is started. Shut-off Valves A and A_1 allow for normal operation of the vacuum system with water flow to the drain.

EXPERIMENTAL PROCEDURE

A nonsteam-injection single-chamber vacuum unit,[1] located ahead of the final heater in a high-temperature short-time pasteurizer, was used to determine the reliability of the water collection system in comparison to Mojonnier total solids and theoretical calculations as a means for determining product loss resulting from vacuum treatment. Mixed raw milk, thoroughly agitated in a storage tank and balance tank, was pasteurized in a 3,000-lb.-per-hour pasteurizer at 172° F. for 16 sec. and homogenized at 2,500 p.s.i. after pasteurization and vacuum treatment. Ten trials, each representing a processing time of 1 hr., were made with a different degree of flash-cooling for each trial, so that flash-cooling increments from 5 to 18° F. were

[1] Vacu-Therm, DeLaval Separator Company, Poughkeepsie, New York.

studied during the trials. The average degree of flash-cooling for each trial was determined from observations (taken at four equally spaced intervals during each trial) of the temperature difference between the milk entering and leaving the vacuum chamber.

The weight of the overflow water collected in the water collection system during each trial, divided by the weight of milk processed, multiplied by 100, resulted in the percentage milk loss. The weight of milk processed per hour was calculated from the average weight of milk collected during four equally spaced 90-sec. flow times from the pasteurizing unit for each hour's run.

The latent heat of vaporization is a constant for respective pressures or vacuua, and radiation loss differences are minor with given equipment when operated at similar capacities. Therefore, the percentage of milk loss based on Mojonnier total solids determinations was calculated using a formula derived from total solids determinations in a previous experiment (2). This formula estimates milk loss from degrees of flash-cooling.

The theoretical percentage milk loss (radiation and convection losses disregarded) was derived from the data in steam tables (1), using the following formula, where h_g is the enthalpy of the saturated vapor and h_f the enthalpy of the saturated liquid:

$$\% \text{ milk loss} = \frac{\text{degrees of flash-cooling} \times 100}{h_g - h_f}$$

The method of least squares was used for determining the linear relationship between the degrees of flash-cooling and percentage milk loss. The standard error of estimate was used as a measure of accuracy of the line and the standard error of the slope as an indication of the accuracy of the slope of the line (3).

RESULTS AND DISCUSSION

A comparison of the water collection system with total solids determinations and theoretical calulations, as a means of determining product loss resulting from nonsteam-injection vacuum treatment of milk, may be noted in Figure 2. The upward trend in the percentage of milk loss, with increasing degrees of flash-cooling on a single vacuum chamber located before final heating, was expressed by the following equations, where Y represents the percentage of milk loss, and X the degrees of flash-cooling:

$$(1)\ Y = -0.174 + 0.096\ X$$

for the water collection system; and

$$(2)\ Y = -0.270 + 0.109\ X$$

for the total solids determinations. The standard error of estimate for the equations was, respectively, 0.03 and 0.19% milk loss. The standard error of the slopes was, respectively, 0.002 and 0.013% milk loss per degree of flash-cooling.

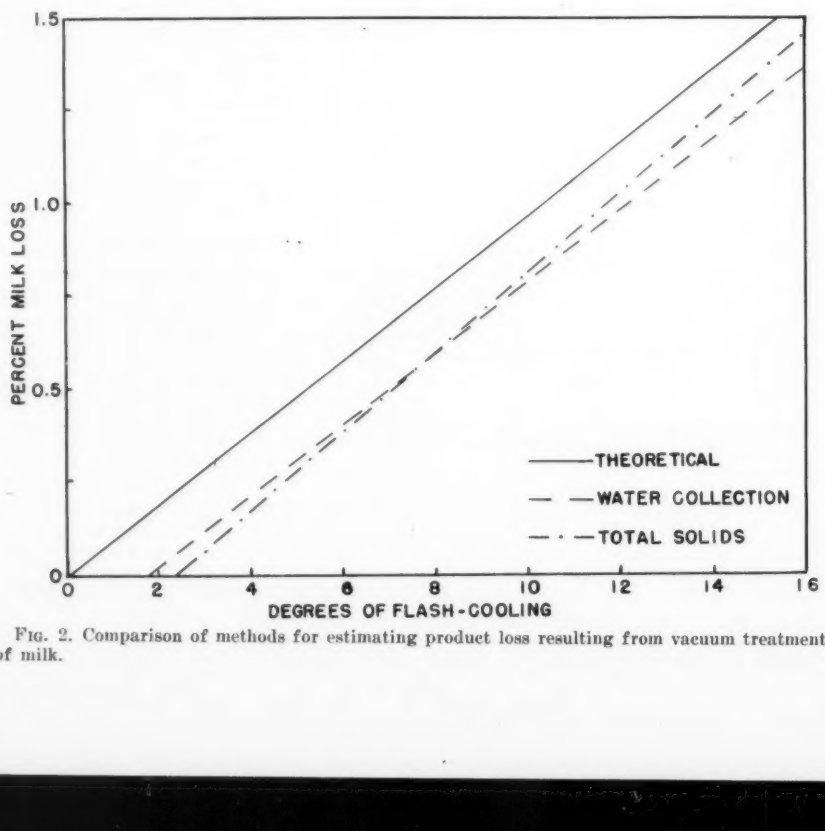

FIG. 2. Comparison of methods for estimating product loss resulting from vacuum treatment of milk.

The water collection line approximately parallels the theoretical line, but intercepts the Y axis at a lower point. This is to be expected since the theoretical line calculations did not include radiation or convection losses. The water collection line should converge on the theoretical line with an increase in the degrees of flash-cooling, since radiation loss will decrease with a decrease in the temperature of the milk leaving the vacuum chamber. However, this convergence is negligible and for practical purposes the water collection line is probably a valid estimate of milk loss. The water collection line does not agree closely with the total solids line. The total solids line converges on the theoretical line with increasing degrees of flash-cooling, but at a lower level of flash-cooling than can be postulated from radiation losses. A slight convergence as degrees of flash-cooling are increased is expected, but not to the extent indicated by the total solids line.

The water collection system appears to be more reliable than total solids determinations as a means of determining milk loss when processing milk in nonsteam-injection vacuum equipment for the following reasons: (1) The results more nearly approximate theoretical values; (2) milk loss estimates are more reproducible, as indicated by the low standard error values for the water collection system data presented in this paper; and (3) estimates of daily or hourly losses are more dependable, since the water collection system includes variations during processing of the milk.

Milk loss estimates can be approximated from degrees of flash-cooling (2), but the water collection system would be useful to dairy plants since it will minimize water usage as well as indicate more accurately product loss with nonsteam-injection vacuum equipment. The system may also be used as a research tool in estimating losses with vacuum treatment and for collection of vapors removed by vacuum treatment for chemical analysis.

ACKNOWLEDGMENTS

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A SMALL-SCALE HEAT EXCHANGER CAPABLE OF HEATING MILK TO 325° F.[1]

For some time we have been interested in such problems as the effect of ultra-high temperatures on the physical and chemical properties of whole and concentrated milks, the thermal destruction of bacteria and bacterial spores, and heat-caused nutritional changes in dairy products. A need for a bench-size heat exchanger, capable of heating milk and concentrated milks to high temperatures in a short period of time, was the incentive to build such an apparatus. Certain performance requirements were decided upon. The heat exchanger should operate at the rate of 1 to 2.5 pt. per minute. It should be capable of heating milk from 40 to 325° F., and to do this in less than 3 sec. In addition, the apparatus must be simple to operate and yet dependable in its performance. A heat exchanger meeting these demands has been made and successfully operated. This unit is pictured in Figure 1 and diagrammed in Figure 2.

The following appears to be maximum heating conditions: pump rate of 2.4 pt. per minute, velocity of 19 ft. per second through the heater, heating time from 40 to 325° F. in 1.9 to 2.0 sec., and cooling time from 325 to 100° F. in 4 to 5 sec. Holding periods at the maximum temperature may be varied by changing the length of ¼-in. O.D. tubing connecting the heater and cooler. Each 4.08 ft. of holding tube will give 1-sec. hold. The accuracy of the controlled temperature appears to be within ± 1° F. Reduction of the pump rate proportionately changes these conditions. In addition to thermocouples (copper-constantan) located at the exit of the heater and cooler, additional thermocouples can be added before the heater and in the holding tube, so that a complete history of the heat treatment can be recorded on the 6-point recorder.

[1] This unit was built with financial support from the United States Steel Corporation.

The critical point in the construction of this equipment was the heater, where it was recognized that a velocity of at least 15-16 ft. per second was essential. Although there appeared to be several heater designs that might be used, the tapered spiral heater was selected to minimize the constriction effects that occur with small amounts of burn-on. The spiral grooves of the heater are 0.10-in. wide and 0.05-in. deep, with three revolutions per inch. The cooler originally served as a heater; however, some cross-over flow was found to occur between the spirals. This fact suggested the tapered design of the heater. Because 1.5-in. wall tubing was not available, the heater was machined from stainless steel castings. All product surfaces are stainless steel.

Pulsations were observed with the duplex pump, so a stainless steel accumulator employing a bladder was made and placed between the pump and heater. This has tended to eliminate the pulsations. To avoid flashing of the liquid in the heater, a back-pressure valve set at 75 p.s.i. was placed at the exit of the cooler. Without cooling water in the cooler, the system can be sterilized to avoid bacterial contamination.

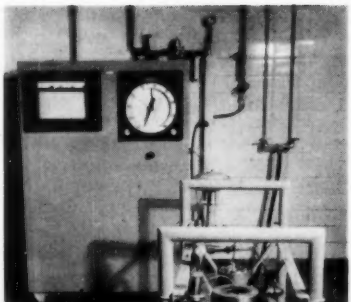

FIG. 1. Front-view photograph of the heat exchanger in operation.

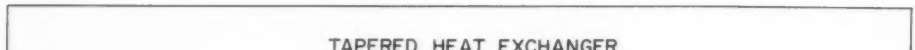

FIG. 2. Flow diagram of the heat exchanger.

The pyrometer is calibrated from 150 to 350° F. with a 2-degree scale. This is the temperature range of the heat exchanger. The 6-point multicolor recorder has a range of 0 to 400° F., with a two-speed drive.

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SPONTANEOUS LIPOLYSIS IN MILK THROUGHOUT THE STAGES OF LACTATION OF INDIVIDUAL COWS

Spontaneous lipolysis has been observed to occur in the milk from individual cows throughout lactation (1–5, 7, 9, 10–19, 21, 22), but its occurrence has been reported to be more frequent late in lactation (1, 3, 4, 5, 11, 14, 17, 19, 22). This type of lipolysis has been termed spontaneous by Tarassuk and Henderson (21), because it requires only cooling and aging to initiate lipolytic action. However, in some of these reports the methods used to evaluate lipolysis were lacking in sensitivity; therefore, total fatty acids liberated were not measured and in others such variables as the changing of feed were not eliminated. Recently developed tests for lipolysis or acid degree values (ADVs),[1] specifically the silica gel method of Harper *et al.* (8), recover the acids freed from the fat by lipolysis more completely than do some of the older methods.

It is well to remember that the terms rancidity and lipolysis are not synonymous. In the opinion of the author, the latter means fat hydrolysis at any level, whereas the former means fat hydrolysis to the extent that it can be detected organoleptically. The purpose of this investigation was to determine the extent of spontaneous lipolysis in a large number of milk samples, from a limited number of individual cows throughout one complete lactation with the silica gel method.

EXPERIMENTAL PROCEDURE

Four Holsteins whose lactation periods started between October 15 and November 10, 1957, were selected. Cows 2, 3, and 4 were in their first lactation and Cow 1 in the second. They were fed a ration of beet pulp, grain, grass silage, and alfalfa hay, but were not allowed access to pasture.

The cows were milked by machine and sampling was started three to four days after parturition. One-half pint portions were taken twice a week from the morning and evening milkings of each cow, and the samples were stored at 4° C. for 20–24 hr. The ADVs were determined by the silica gel method (8). All of the samples were checked organoleptically. Records were kept of production, butter fat content (Babcock), and unusual occurrences, such as illness and estrus.

RESULTS

As is illustrated in Figures 1 and 2, Cows 3 and 4 produced milk with greater susceptibility to spontaneous lipolysis in the later stages of lactation than did Cows 1 and 2. Each point on the graphs represents the average ADV of two morning and two evening samples. In general, except for Cow 1, the ADVs increased as lactation progressed. None of the samples from

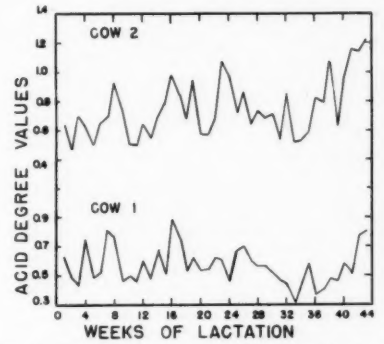

FIG. 1. Acid degree values (silica gel) of milk samples for complete lactations. Each point is the average of two morning and two evening samples. Acid degree value is the milliliters of 1 *N* alkali required to neutralize the acidity of 100 g. of fat.

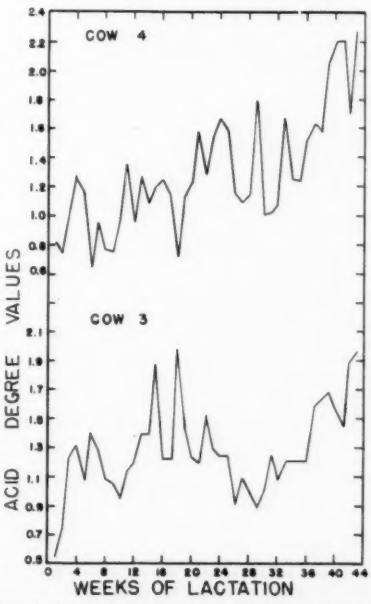

FIG. 2. Acid degree values (silica gel) of milk samples for complete lactations. Each point is the average of two morning and two evening samples. Acid degree value is the milliliters of 1 *N* alkali required to neutralize the acidity of 100 g. of fat.

[1] Acid degree value, or ADV, is the number of milliliters of *N* alkali required to neutralize the acidity of 100 g. of fat.

Cows 1 and 2 were rancid organoleptically. Nine of the 12 samples from Cow 3 which tasted rancid were taken during the last month of lactation and with Cow 4, 11 of 15 were obtained in the last month. All of the individual samples with a rancid flavor had an ADV of 1.90 or higher.

Table 1 shows that the average ADVs of Cows 3 and 4 were higher than the averages of Cows 1 and 2. As Table 1 illustrates, the average ADVs of the evening samples were higher than the averages of the morning samples, except for Cow 1. The differences were significant with Cows 2 and 3 when analyzed by the "t" test, but the average evening value for all cows was not significantly higher than the average morning values.

TABLE 1

Statistical data. Acid degree values (ADVs) of milk from cows throughout the stage of lactation

Cow No.	1	2	3	4
Av. ADV	0.57	0.75	1.25	1.28
Av. A.M. ADV	0.57	0.67	1.18	1.25
Av. P.M. ADV	0.56	0.80	1.31	1.30
"t" test				
A.M.-P.M.	0.1	3.522	2.263	0.9592
P	—	0.001	0.025	—

DISCUSSION

The data reported herein obtained by the silica gel ADV method confirm the work of previous investigators (5, 9, 10, 12, 13, 15, 18, 19), in that the susceptibility to lipolysis is subject to considerable variation from cow-to-cow as well as within cows over a complete lactation. Cow 1, which did not produce rancid milk and with the least variation in ADVs was a second-lactation cow, which is suggestive, but the limited number of cows studied does not permit conclusions.

The higher average ADVs of the evening milkings, as compared to the ADVs of the morning milkings, are difficult to explain, but for individual cows the difference may be valid, since a large number of samples were taken.

Figures 1 and 2 show that the weekly variation even in pooled samples was great, which illustrates the importance of cow individuality when dealing with spontaneous lipolysis. There are many points on the curves where the high ADVs can not be explained by stage of lactation or feeding, thus suggesting that unrecognized factors are operating. Tarassuk and Frankel (20) state that the lipase causing spontaneous lipolysis varies from a low concentration in normal milk to a very high concentration in naturally active milk. This may explain the individual variations noted in the present study. The explanations for the variations in lipase concentration are as yet unknown.

SUMMARY

The milk from each of four cows maintained under controlled conditions was checked for spontaneous lipolysis four times a week during a complete lactation by the silica gel method. The average acid degree values (ADVs) were: Cow 1—0.57, Cow 2—0.75, Cow 3—1.25, and Cow 4—1.28. The ADVs increased as lactation progressed with Cows 2, 3, and 4. Samples that were rancid organoleptically were produced only by Cows 3 and 4. Most of these samples were taken during the last month of lactation. There was considerable week-to-week and cow-to-cow variation. The average P.M. ADVs of Cows 2 and 3 were significantly higher than the average A.M. ADVS.

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OUR ASSOCIATION

ERRATA

CRITICAL FEATURES OF GOOD DAIRY FEEDING EXPERIMENTS

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Department of Experimental Statistics, North Carolina State College, Raleigh

Errata sheets for the mimeographed material distributed at the 54th Annual Meeting of the American Dairy Science Association are now available. In addition to a number of minor mistakes, a serious error was made in the construction of two of the tables in the Appendix of the handout. This error led to misleading answers for the problems in the Appendix that are based on the two tables.

In view of the serious nature of this error, the author would greatly appreciate having all who received the mimeographed material at the Annual Meeting write immediately for errata sheets.

BOOK REVIEW

THE UNITED KINGDOM DAIRY INDUSTRY. E. L. Crossley. Richard Clay and Company, Ltd., Bungay, Suffolk, England. 142 pp. 1959.

Professor E. L. Crossley of the University of Reading, with the help of 16 collaborators, has compiled an attractive book on the growth of the dairy industry in the United Kingdom during the past 30 years. This book is profusely illustrated with photographs, figures, and tables, a special feature being 22 colored pictures of the British breeds of cattle and of cheeses.

An attempt has been made to present a broad outline of the dairy industry in the United Kingdom, which consists of England, Wales, Scotland, and Northern Ireland, the Isle of Man and several other small islands. The United Kingdom has a land area of about 93,360 sq. mi., divided as follows: England 53.9%, Wales 8.6%, Scotland 31.9%, and Northern Ireland 5.6%. This area supports 51 million people, with 42 million in England, Wales 2.6 million, Scotland 5.1 million, and Northern Ireland 1.4 million. Out of this population there are 300,000 farmers and 698,000 agricultural workers. It is this land area, with the production of its workers, about which Professor Crossley tells his story of progress in the dairy industry. Both the production and consumption of milk have increased greatly. Traditional farm-house dairying has passed away, replaced with a modern industry of high technological development. The processing of fluid milk and distributing it to every family in the country are outstanding technological achievements of the dairy engineering industry, based upon research and education.

This small densely populated country has developed one of the great dairy industries in the world. Professor Crossley and his collaborators tell a most interesting story. This is a very attractive and readable book.

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University of Illinois
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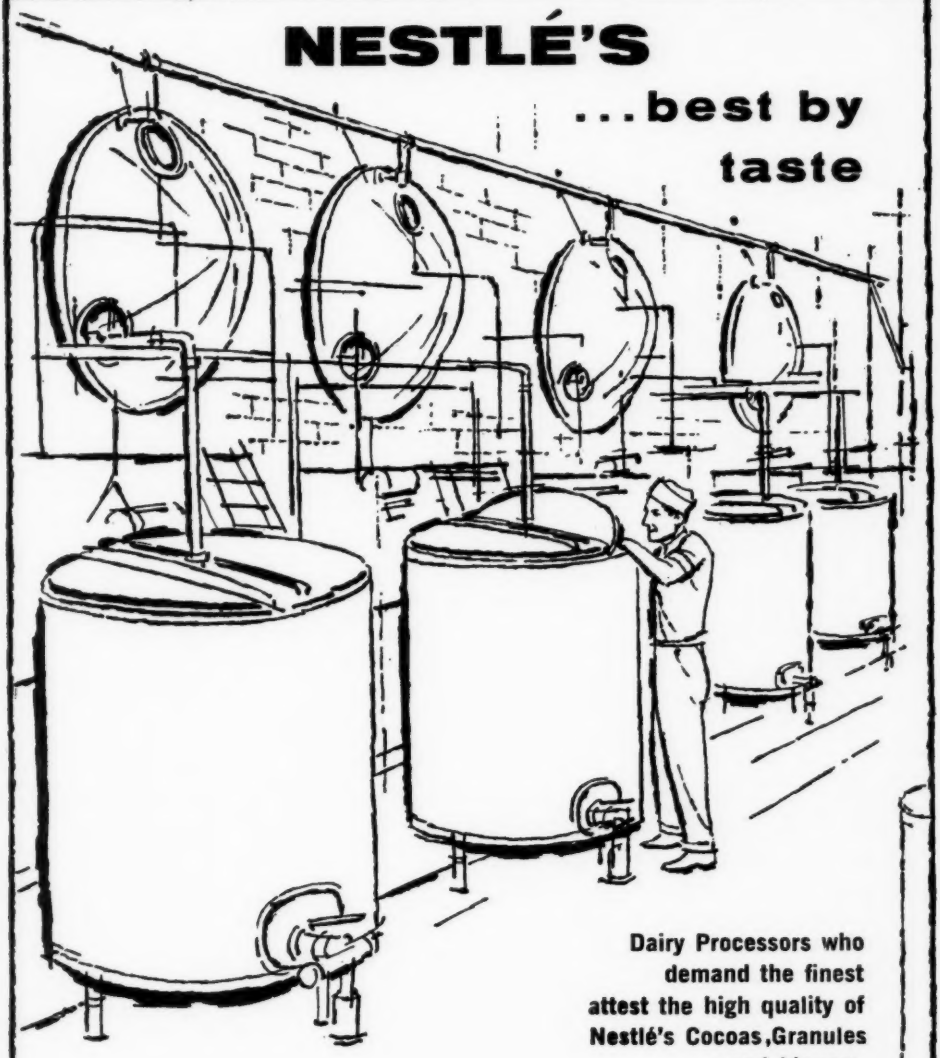

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